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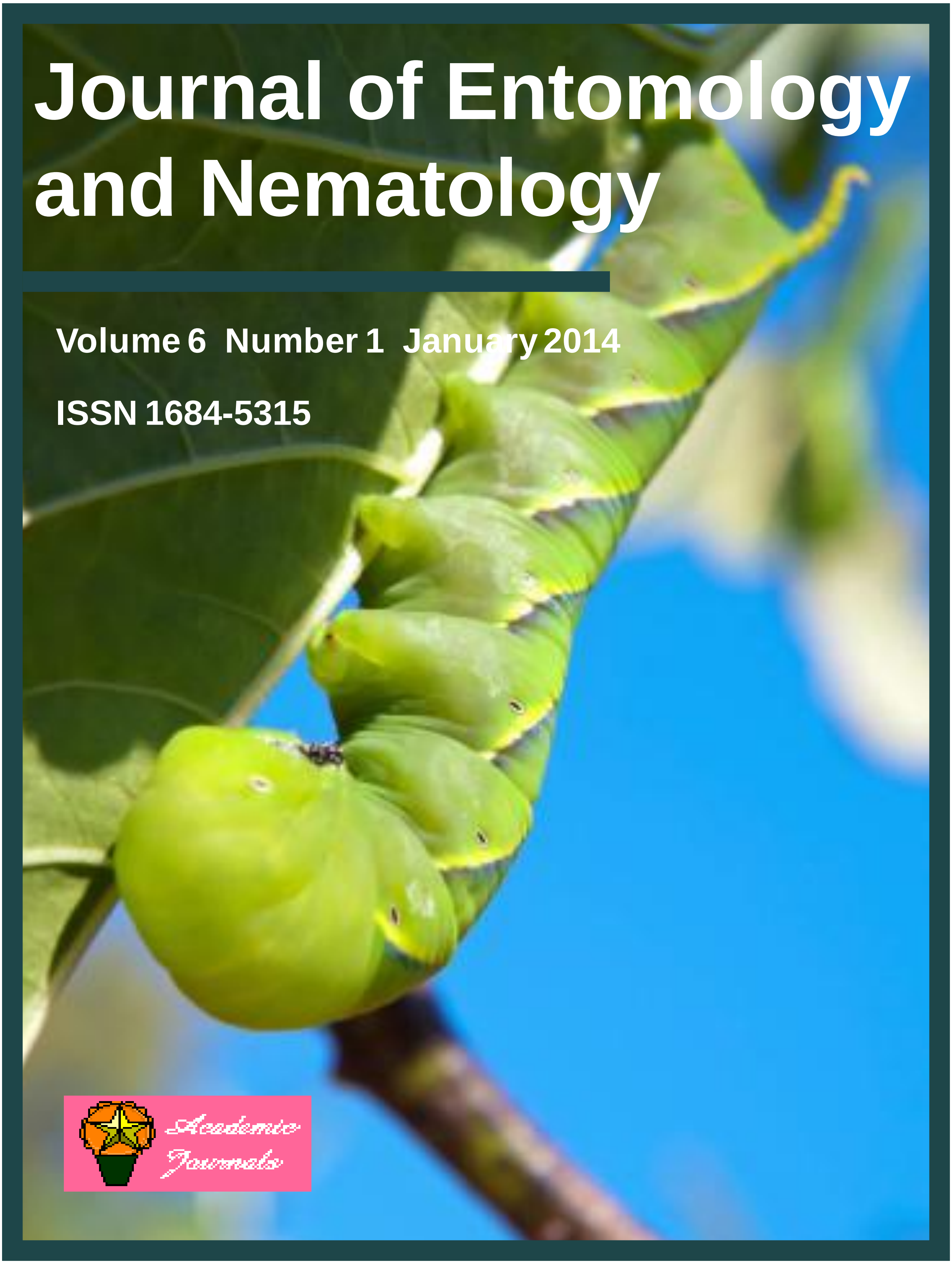

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Full Length Research Paper

Distribution and impact of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and C*hilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in Northeastern Ethiopia

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An assessment was done during the long and short rainy cropping seasons of 2010/2011 across different agro-climatic zones (ACZs) with the objective of determining the distribution, species composition and damage levels of important stem borer species in North-eastern Ethiopia. Data were collected from 21 localities of six districts, four fields in each locality, and a total of 84 fields were assessed. *Busseola fusca* composed of 16 to 100% of the population of stem borer species and caused damage levels of 3 to 60% in South Wollo zone at the elevation ranging from 1750 to 2338 m. Moreover, *Chilo partellus* is composed of 7 to 100% of the population of stem borer species and cause a damage of 1 to 100% in the same zone at the elevation range of 1492 to 2084 m. Similarly, in North Wollo zone, *B. fusca* shared 69 to 88% of the total population of stem borer species and cause damage levels of 5 to 53% of the elevation ranging from 1850 to 2044 m, while *C. partellus* composed of 12 to 31% and caused damage of 2 to 26%. In the Oromia administrative zone, which falls at the elevation of 1400 to 1669 m, 100% of the stem borer population was *C. partellus* and caused a damage level of 84 to 99%. The result indicates that *C. partellus* widened its distribution and extent of damage from the previous report of 1900 up to 2044 m. The two stem borers were found in elevation ranging from 1750 to 2044 m but their level of distribution, compositions and damage varied between elevations. Conclusively, *C. partellus* widened its distribution and might have replaced the indigenous species, because it was recorded as up to 2044 m, which was not reported in the previous decades.

Key words: Stemborers, elevation, agro-ecology, composition.

INTRODUCTION

Lepidopteran stem borers are generally considered to be the most damaging insect pests of maize and sorghum in Africa (Seshu, 1998). Maes (1998) reported 20 economically important stem borer species whose distribution, relative abundance and pest status are expected to vary with environmental conditions. *Busseola fusca* and the exotic *Chilo partellus* are the dominant species in East Africa. *C. partellus* has proven to be a highly competitive colonizer in many areas of eastern and southern Africa, often becoming the most injurious stem borer and displacing native species (Kfir, 1997). Studies in coastal Kenya showed that *C. partellus* has partially displaced the indigenous stem borer, *Chilo orichalcociliellus* (Lepidoptera: Crambidae) (Ofomata et al., 1999). In the

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Eastern province of Kenya, *C. partellus* although present in the early 1980s, it was less abundant than *B. fusca* (Seshu, 1983). However, in the same area in the period 1996 to 1998, *B. fusca* became rare and *C. partellus* became dominant (Songa, 1999). Similarly, in the eastern highveld region of South Africa, *C. partellus* partially displaced *B. fusca* over a period of seven years (Kfir, 1997). The displacement was most evident in grain sorghum where the proportion of *C. partellus* in the total stem borer population increased from about 3% in 1986 to 1991% in 1992 (Kfir, 1997).

Since the appearance of *C. partellus* in African continent in 1932, it has continuously expanded its distribution in the warm, low-altitude regions of eastern and southern Africa (Kfir, 1997). The same author reported that *C. partellus* was expanding its distribution into the high elevations of the eastern highveld region of South Africa. The only stem borer already found at an elevation of 1,600 m was *B. fusca*. After *C. partellus* invaded this region, it rapidly increased its share of the total borer population every year. In maize, it reached 32% of the total borer population within six years and on grain sorghum, 59% within seven years. Within two years, it became the predominant borer; constituting 90% of the total stemborer population. One of the possible reasons for the replacement of the indigenous species is hibernating for larval populations of *C. partellus* terminate diapause and emerge in one month earlier than *B. fusca.* This enables *C. partellus* to infest the grain sorghum before *B. fusca,* thus becoming the predominant borer in this niche. In addition, the life cycle of *C. partellus* is three weeks shorter than that of *B. fusca,* which gives it a further competitive advantage because of its higher potential rate of an increase (Kfir, 1997). Emana et al. (2008) reported from his survey that *B. fusca* was the dominant stem borer species in high-potential zones (highland tropics, moist transitional zone and moist mid-altitude) while the exotic *C. partellus* dominated smallholder farms in low potential zones (dry mid altitude, dry transitional and lowland tropical zone). In India, Gupta et al. (2010) reported that *C. partellus,* one of the most destructive pests of maize and sorghum, is the most important at altitudes below 1500 m above sea level.

In Ethiopia, *B. fusca* and *C. partellus* are considered to be the most damaging insect pests, with reported yield losses of 0 to 100, 39 to 100, 10 to 19 and 2 to 27% from South, North, East and Western Ethiopia, respectively (Melaku and Gashawbeza, 1993; Melaku et al., 2006).

Previous two decades, Assefa (1985) reported that *C. partellus* was a predominant species at lower elevation of less than 1700 m and *B. fusca* was dominant at high elevation of 1160 - 2600 m.a.s.l. and in cooler areas. Emana et al. (2001) conducted a survey in 1999 and 2000 and reported that *C. partellus* widened its distribution from 500 - 1700 to 1030 - 1900 m.a.s.l. whereas *B. fusca* was recorded between 1030 - 2320 m.a.s.l. However, studies were conducted in different on the compositions, distribution and damage levels of these stemborer species in northeastern Ethiopia. Thus, understanding species distribution and abundance of stem borer communities will constitute basic information necessary for future development of management strategies. Therefore, the objective of this study was to know species composition, levels of damage and status of the most important stem borer species at different agro-ecological zones of northeastern part of Ethiopia.

MATERIALS AND METHODS

Survey sites descriptions

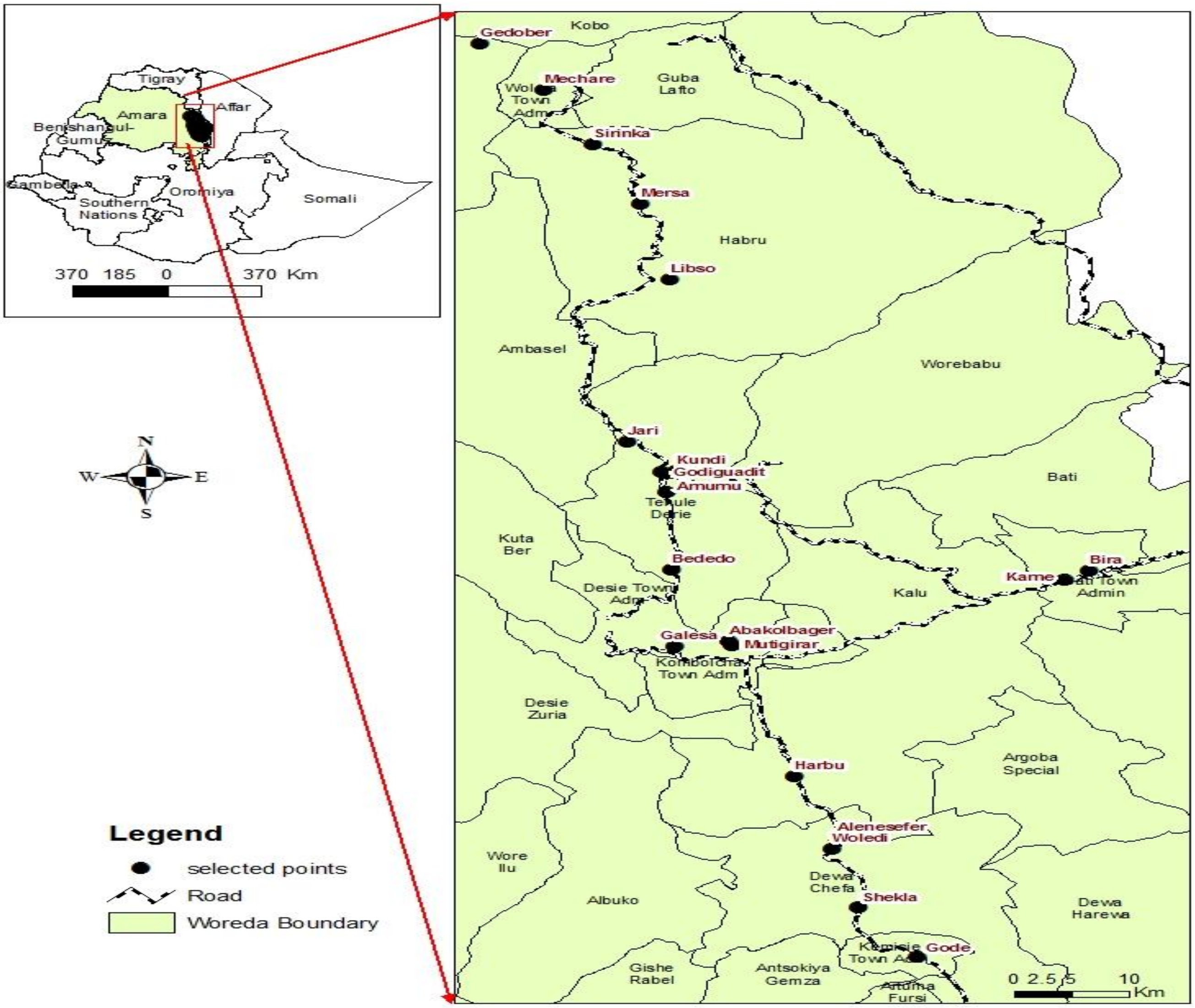
Field surveys were conducted in North Wollo, South Wollo and Oromia zones of northeastern Ethiopia in 2010/11. The six randomly selected districts were Tehulederi and Kalu (39°40 - 43'E and 11°6 - 19'N) (South Wollo); Habru (39°31 - 39'E and 11°40 - 52'N) and Gubalafto) (North Wollo) and Dawa Chefa (39°48'E and 10°51'N) and Bati (39°59'E and 11°11'N) (Oromia) (Map 1).

Rain fed sorghum and irrigated maize is practiced in all districts. The study areas experience bimodal rainfall, the short rainfall in April or May and main rain June to September. Late and early maturing sorghum cultivars are planted in the short and main rainfall seasons, respectively. During the study period, all fields were covered with late maturing sorghum cultivar, *Degalit*. Based on the classification of agro-ecological zones (AEZs), almost all the zones experienced sub-moist warm (Kola, <1,500 m), dry-warm to moist cool (Woinadega, 1,500 - 2,500 m) and moist cool (Dega, 2,500 - 3,500 m) (MoA, 2000) (Table 1).

Sampling procedures

Sampling procedures were done following the work of Emana (2001, 2008). Randomly, selected 3 to 4 peasant associations (PAs) were considered from each district. Four well managed farmers' fields (chemical untreated) with a minimum size of one hectare were selected. The fields were selected on the basis of accessibility and area coverage of sorghum and maize. A total of 84 fields from 21 PAs was assessed (Table 1). In each field, five plots with 3 × 3 m sizes were sampled in 'Z' fashion.

The assessments were conducted in seedling, booting and harvesting stages of sorghum and at tasseling of irrigated maize. The same fields were used for different time of sampling. The presence of the two stemborer species in the same field was confirmed at the seedling stage of sorghum. The presence of the two stemborers in the same field at seedling stage was confirmed using their oviposition sites on the plants. Egg oviposition sites of the two species are on different parts sorghum. Female *B. fusca* laid eggs between the leaf sheath and stem whereas *C. partellus* lay eggs on the undersides of leaves, mainly near the midribs. In fields where both the stem borers were found, all the plants in the plots were counted, dissected and data on the number of plants with leaf damage and larval density were collected. It was rare to find the two species on the same plant. In fields where a single species was found, six plants per plot were dissected and similar types of data were collected. At harvest, in each plot the total number of stems, and damaged stems were counted. The severity of leaf damage on sorghum/maize due to the two stem borer species was based on the assessment of the amount of feeding by the insects on the upper leaves in each plot at seedling (small size larvae), booting (medium size larvae) and harvest (large size larvae) stage of sorghum and at tasseling stage of maize. The leaf feeding damage parts of the country, there are no exhaustive information was scored on a 0 - 5 scale, where 0 = 0 (no damage symptom), 1 = 1 - 20%;



Map 1. Map of northeastern Ethiopia showing localities surveyed in the three zones.

2 = 21-40%; 3 = 41-60%; 4 = 61-80% and 5 = over 81% damage or complete death of the plant (Kalule et al., 1997).

The proportion of infested plants (IP) was calculated in relation to total sampled plants (TP). IP (%) = $\frac{IP}{TP}$, where, IP = infested plants, TP = total plants in a quadrant.

The larvae and adults of *B. fusca* and *C. partellus* were identified using identification keys, color pictures and morphological differences. *C. partellus* larvae have a cream to pink coloration, with dark spots along the dorsal surface; the head capsule is brown. Moreover, *C. partellus* larvae can be distinguished from *B. fusca* by the presence of a complete circle crochets on the prolegs whereas in *B. fusca* the crochets are arranged in a crescent (Hutchison et al., 2008). The field identification was confirmed by rearing fifty of the identified larvae per plot to the adult stage in the laboratory. Adult moth identification was carried out. The selected maize fields were sown from December to January using irrigation and sorghum in April/May using short rain period. In all districts, farmers practiced sowing maize under irrigation. Fields in all the surveyed districts were covered by *Degalit* and all the data in this study were from this cultivar. Data on temperature (maximum and minimum), rainfall (mm) and relative humidity (%), of each locality were obtained from the nearby meteorological stations. However, few stations do not have data on relative humidity, and they call it third class stations (Table 1). Coordinates and elevations of each field were recorded using global positioning system (GPS) 2000-2007 with brand name Garmin Ltd or its subsidiaries.

Data analysis

The data were arranged in nested design and analyzed using SPSS Version 12 software. The significantly different means (<0.05) were separated using Student-Newman-Keuls (SNK) multiple range test (Gomez and Gomez, 1984; Hinkelmann and Kempthorne, 2008). To normalize the data, Arcsin and square root transformations were used for percentage and count data, respectively, and distrasformed thereafter. Correlations of different data were also analyzed.

Table 1. Elevation (m), temperature (°C), relative humidity (%) and rainfall of each locality of the three zones of northeast Ethiopia in 2010.

Zones/PAs	Elevation (m)	Annual rainfall (mm)	Mean temperature (°C)		Mean relative humidity (%)
			Maximum	Minimum	
South Wollo					
Bededo	2291 - 2338	1899.2	21.6	9.6	na
Godiguadit	1887 - 1911	1899.2	25.6	10.6	na
Jari	1680 - 1750	1479.5	27.2	11.4	na
Amumu	1960 - 1970	1835.3	26.3	10.6	na
Abakolbager	1841 - 1857	1313.5	26.3	13.2	na
Mutigirar	1834 - 1842	1313.5	26.2	13.2	65.2
Galesa	1923 - 2084	1426.4	24.6	11.3	71.0
Harbu	1492 - 1527	1220.8	31.8	12.8	55.8
North Wollo					
Gedober	1885 - 1980	1285.3	26.2	13.2	na
Mechare	1870 - 2044	1396.2	25.2	11.5	na
Jarsa	1856 - 1980	1396.2	27.7	11.5	Na
Libso	1508 - 1670	997.2	29.1	14.0	na
Mersa	1595 - 1670	1244.3	28.8	13.7	na
Sirinka	1850 - 1889	1199.8	26.5	13.1	62.6
Oromia					
Woledi	1640 - 1669	1027.3	29.9	12.7	64.0
Shekla	1432 - 1669	1027.3	29.9	12.7	60.3
Gode	1419 - 1431	1375.2	30.7	14.6	55.7
Alenesefe	1471 - 1490	1027.3	29.9	12.7	64.0
Kame	1555 - 1576	1183.0	28.3	13.6	67.2
Bira	1640 - 1657	1183.0	28.3	13.6	67.2
Fura	1412 - 1515	1183.0	28.3	13.6	67.2

na = data not available.

RESULTS

Density, damage and composition of stemborers on sorghum and maize

South Wollo

The indigenous *B. fusca* and the invasive *C. partellus* were the most injurious stemborers recorded on sorghum and maize. The larval density of *B. fusca* and *C. partellus* varied between elevations and crop stages. Thus, the mean numbers of *B. fusca* larvae per plant were 1 - 4, 2 - 4 and <1 in elevation >2291, 1887 and 1750 m.a.s.l, respectively (Figure 1). The larval density of *B. fusca* was higher at booting stage than the two growth stages of sorghum (Figure 2). *B. fusca* caused maize and sorghum damage of 12 to 60 and 14 to 51% in elevations ranging from 1887 to 2338 m.a.s.l. However, *C. partellus* caused maize and sorghum damage by 1 to 4 and 4 to 23% in the same elevations.

High numbers of *C. partellus* larval density (>10 larvae/plant) was recorded at lower elevations between 1680 - 1750 m.a.s.l and on sorghum at seedling stage. *B. fusca* and *C. partellus* were not recorded in elevations 1750 and >2291 m.a.s.l. of the same district, respectively. *C. partellus* caused significantly (P <0.05) high maize and sorghum damage (80 to 100%) in elevation <1750 m (Table 2). In this district, the two species were found in elevation between 1887 and 1970 m.a.s.l with a proportion of 65-89 (*B. fusca)* and 11 to 35% (*C. partellus)* on both crops (Table 2).

B. fusca was the dominant species and shared 65 to 100% in the highland areas (1887 - 1970 m.a.s.l) with mean minimum and maximum temperatures of 4.5 - 14 and 19.5 - 27°C, respectively. In contrast, *C. partellus* was dominant and shared 100% in lowland areas (<1750 m.a.s.l.) with mean minimum and maximum temperatures of 7-15 and 25-32.5°C, respect-tively Table 2). Damage severity scores (1-5 scale) of 1-3 (20 to 45%) (*B. fusca*) and 1-2 (20-25%) (*C. partellus*) on maize and sorghum were recorded, respectively. Maize and sorghum were severely damaged by *C. partellus* at an elevation

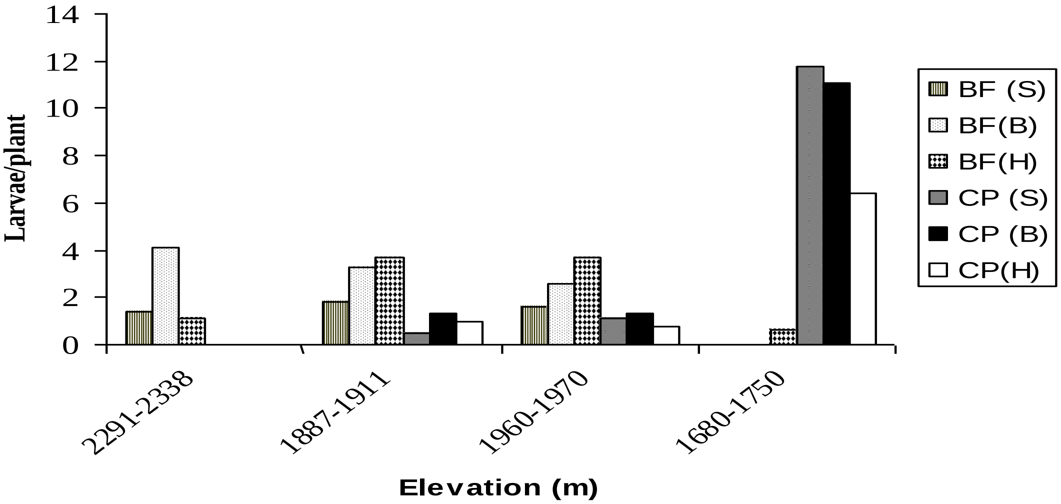


Figure 1. Larval density of *B. fusca* and *C. partellus* at different sorghum growth stages (S = seedling, B = booting, H = harvesting) at Tehulederi District, south Wollo administrative zone in 2010/11.

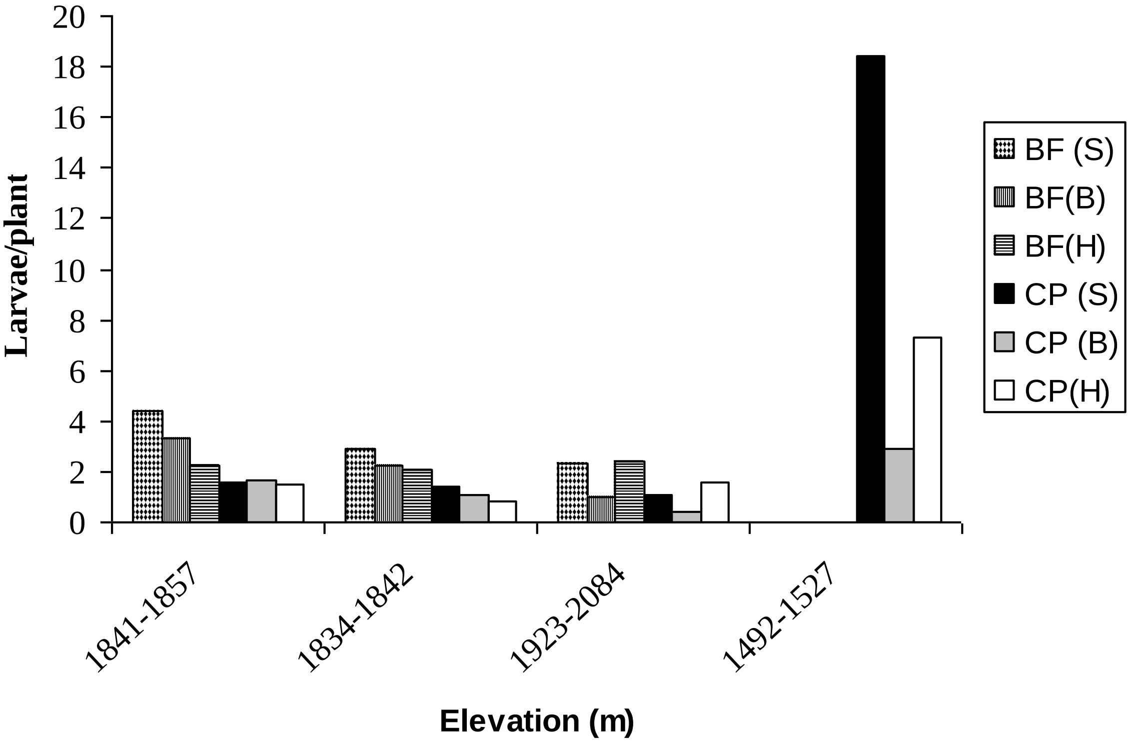


Figure 2. Larval density of *B. fusca* and *C. partellus* at different sorghum growth stages (S = seedling, B = booting, H = harvesting) at Kalu district, south Wollo administrative zone in 2010/11.

between 1680-1750 m.a.s.l. with damage scales of 4 - 5 (61 to 100%) (Table 3).

Percent damage and number of *B. fusca* larvae had weak positive correlation with elevation with r = 0.2, P = 0.663 and r = 0.1, P = 0.083, respectively. However, percent damage and number of *C. partellus* larvae had significant high inverse relation with elevation with r = -0.76, P<0.001 and r = -0.71, P<0.002, respectively. Moreover, percent damage and the number of larvae had positive correlation with r = 0.82, P <0.001 (*B. fusca*) and r = 0.89, P < 0.001 (*C. partellus*). In addition, temperature and composition of *C. partellus* and *B. fusca* had positive

Table 2. Mean (± SE) infestation (%) of maize and sorghum due to the two stem borer species in South and North and Wollo zones in 2010/11.

District	Alt	Mean (± SE) infestation (%)							
		Maize		Sorghum					
				Seedling		Booting		Harvesting	
		BF	**CP**	**BF**	**CP**	**BF**	**CP**	**BF**	**CP**
Tehulederi (SW)	2291 - 2338	11.6 ± 3.9^{b}	0.0 ± 0.0^{c}	13.5 ± 4.8^{b}	0.0 ± 0.0^{c}	20.9 ± 1.7^{a}	0.0 ± 0.0^{c}	13.5 ± 1.4^{bc}	0.0 ± 0.0d
	1960 - 1970	59.7 ± 4.4^{a}	3.5 ± 2.4^{b}	46.0 ± 9.3^{a}	11.4 ± 6.6^{b}	14.1 ± 2.4^{b}	3.7 ± 2.2^{b}	23.2 ± 2.4^{b}	8.5 ± 2.5^{c}
	1887 - 1911	48.0 ± 7.3^{a}	1.2 ± 0.9^{c}	40.9 ± 6.4^{a}	23.0 ± 2.6^{b}	24.8 ± 9.4^{a}	3.9. ± 2.2^{bc}	51.0 ± 7.0^{a}	23.0 ± 2.8^{b}
	1680 - 1750	0.0 ± 0.0^{c}	79.8 ± 1.7^{a}	0.0 ± 0.0^{c}	100 ± 6.6^{a}	0.0 ± 0.0^{c}	95.5 ± 2.1^{a}	2.5 ± 1.5^{c}	93.5 ± 1.6^{a}
	F	16.3	643.5	12.8	161.6	5.0	876.9	29.5	440.4
	P	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
	df	15	15	15	15	15	15	15	15
Kalu (SW)	1923 - 2084	13.7 ± 3.9^{b}	4.0 ± 1.0^{b}	30.8 ± 3.5^{ab}	3.1 ± 1.0^{c}	14.4 ± 1.1^{b}	1.0 ± 0.5^{c}	43.4 ± 3.7^{a}	13.9 ± 2.0^{b}
	1841 - 1857	16.5 ± 4.4^{b}	3.3 ± 1.8^{b}	43.7 ± 4.7^{a}	13.7 ± 2.3^{b}	43.9 ± 4.5^{a}	8.6 ± 1.4^{b}	56.4 ± 6.4^{a}	15.1 ± 3.0^{b}
	1834 - 1842	41.9 ± 7.1^{a}	5.6 ± 2.1^{b}	26.6 ± 6.2^{b}	7.3 ± 3.4^{bc}	30.4 ± 9.3^{a}	5.9 ± 1.8^{b}	44.1 ± 5.5^{a}	16.7 ± 2.8^{b}
	1492 - 1527	0.0 ± 0.0^{c}	89.9 ± 5.7^{a}	0.0 ± 0.0^{c}	94.6 ± 2.3^{a}	0.0 ± 0.0^{b}	94.1 ± 1.1^{a}	0 ± 0^{b}	98.7 ± 0.8^{a}
	F	11.4	141.9	18.5	322.6	16.2	1225.7	28.9	328.3
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	df	15	15	15	15	15	15	15	15
Gubalafto (NW)	1885 - 1980	53.2 ± 8.1^{a}	11.9 ± 1.2^{a}	19.8 ± 1.8^{a}	4.2 ± 1.7^{a}	30.0 ± 6.3^{a}	11.1 ± 5.4^{b}	17.6 ± 2.0^{a}	4.5 ± 1.1^{a}
	1870 - 2044	25.7 ± 4.2^{b}	3.9 ± 1.1^{b}	26.8 ± 2.8^{a}	4.1 ± 3.0^{a}	38.2 ± 6.2^{a}	25.8 ± 5.7^{a}	4.8 ± 1.3^{b}	1.6 ± 0.4^{b}
	1856 - 1980	23.1 ± 4.6^{b}	3.2 ± 1.2^{b}	21.7 ± 2.4^{a}	10.3 ± 4.8^{a}	20.9 ± 4.1^{a}	14.4 ± 2.0^{b}	18.3 ± 0.9^{a}	8.7 ± 1.0^{a}
	F	9.1	19.1	1.4	1.1	2.2	2.7	27.3	16.9
	p	0.002	0.000	0.11	0.12	0.28	0.04	0.001	0.004
	df	15	15	15	15	15	15	15	15
Habru (NW)	1850 - 1889	37.0 ± 8.0^{a}	13.8 ± 6.9^{b}	33.9 ± 7.5^{a}	4.5 ± 2.4^{c}	46.8 ± 6.9^{a}	21.4 ± 0.4^{b}	74.6 ± 3.4^{a}	15.1 ± 1.7^{b}
	1595 - 1670	0.0 ± 0.0^{b}	69.8 ± 4.5^{a}	0.0 ± 0.0^{b}	75.6 ± 3.4^{b}	0.0 ± 0.0^{b}	94.6 ± 2.6^{a}	0.0 ± 0.0^{c}	92.4 ± 1.1^{a}
	1508 - 1670	0.0 ± 0.0^{b}	93.5 ± 6.2^{a}	0.0 ± 0.0^{b}	95.8 ± 4.1^{b}	0.0 ± 0.0^{b}	95.1 ± 2.8^{a}	0.0 ± 0.0^{c}	91.0 ± 1.9^{a}
	F v^{a}lue	21.3	26.9	20.4	200.4	46.5	601.9	209.0	616.8
	P v^{a}lue	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	df	15	15	15	15	15	15	15	15

Bf = *Busseola fusca*, *Cp* = *Chilo partellus*, mean (± SE) within columns, along each district, followed by the same letters do not differ significantly at the 5% threshold (Student-Newma n-Keuls-SNK).

and negative correlations with r = 0.50, P = 0.501 and r = -0.97, P = 0.030, respectively.

In Kalu district, high *B. fusca* larval density (>4 larvae/plant) was recorded at the seedling stage and at an elevation between 1841 and 1857 m.a.s.l. Lower number of *B. fusca* larvae/plant were recorded at harvesting than at booting stage of sorghum. However, higher *C. partellus* larvae (>18 larvae/plant) were recorded in low-land areas (1492-1527 m.a.s.l.) and at seedling stage (Figure 3). *B. fusca* caused maize and sorghum damage by 13 to 41 and 14 to 56%, respect-tively in elevations ranging from 1834 to 2084 m.a.s.l. However, *C. partellus* caused maize and sorghum damage by 3-6 and 4-17%, respectively, in the same elevations. *C. partellus* caused significantly (P <0.05) high maize and sorghum damage (90-99%) in elevation between 1492 and 1572 m.a.s.l. (Table 2). In this district, the two species were found in elevation between 1834 and 2084 m.a.s.l. with proportion of 65 to100% (*B. fusca*) and 7 to 42% (*C. partellus*) on both crops (Table 3). Damage severity scores (1-5 scale) of 1-3 (20 to 45%) (*B. fusca*) and 1-3 (20 to 30%) (*C. partellus*) on maize and sorghum were recorded, respect-tively. Maize and sorghum were severely damaged by *C. partellus* at elevation between 1492 to 1527 m.a.s.l. with damage scales of 4-5 (61 to 100%) (Table 3). Generally, in South Wollo, relatively high percentage damages (15 to 45%) of sorghum and maize due to *B. fusca* were recorded except some localities with lower elevations (Table 4). However, low percentage damages (<15%) due to *C. partellus* were recorded in highland areas of this zone except two areas with low elevations.

In Kalu district, percent damage and number of larvae of *B. fusca* had positive correlation with elevation with r = 0.38, P = 0.001 and r = 0.73, P = 0.001, respectively. In

Table 3. Mean (-SE) damage score (1-5 scale) of the two stemborer species on sorghum and maize South and North Wollo zones in 2010/11.

District	Alt	Mean (± SE) Score (1-5 scale)							
		Maize		Sorghum					
				Seedling		Booting		Harvesting	
		BF	CP	BF	CP	BF	CP	BF	CP
Tehulederi (SW)	2291 - 2338	1.5 ± 0.3^{b}	0.0 ± 0.0^{b}	1.3 ± 0.1^{c}	0.0 ± 0.0^{b}	2.0 ± 0.0^{b}	0.0 ± 0.0^{b}	1.1 ± 0.1^{b}	0.0 ± 0.0^{bc}
	1960 - 1970	3.1 ± 0.3^{a}	2.0 ± 1.1^{b}	3.2 ± 0.1^{a}	1.9 ± 1.1^{b}	1.5 ± 0.4^{b}	1.0 ± 0.1^{b}	1.5 ± 0.2^{b}	0.9 ± 0.1^{b}
	1887 - 1911	3.3 ± 0.3^{a}	1.5 ± 0.7^{ab}	2.5 ± 0.0^{b}	1.5 ± 0.0^{a}	2.4 ± 0.4^{a}	1.0 ± 0.4^{b}	2.5 ± 0.2^{a}	1.5 ± 0.2^{b}
	1680 - 1750	0.0 ± 0.0^{b}	4.0 ± 0.3^{a}	0.0 ± 0.0^{c}	4.5 ± 0.0^{a}	0.0 ± 0.0^{c}	5.0 ± 0.0^{a}	1.0 ± 0.1^{b}	4.7 ± 0.4^{a}
	F	58.4	14.9	196.0	16.9	10.1	21.1	23.1	35.5
	P	0.04	0.02	0.00	0.01	0.04	0.00	0.04	0.001
	df	15	15	15	15	15	15	15	15
Kalu (SW)	1923 - 2084	1.0 ± 0.3^{b}	3.0 ± 0.3^{b}	2.0 ± 0.6^{a}	3.0 ± 0.0^{b}	3.0 ± 0.3^{a}	2.0 ± 0.4^{b}	$3.0 \pm {}^{a}$	2.0 ± 0.2^{b}
	1841 - 1857	2.0 ± 0.3^{b}	2.0 ± 0.6^{b}	3.0 ± 0.3^{a}	3.0 ± 0.0^{b}	3.0 ± 0.3^{a}	2.0 ± 0.5^{b}	$3.0 \pm {}^{a}$	2.0 ± 0.3^{b}
	1834 - 1842	3.0 ± 0.0^{a}	2.0 ± 0.4^{b}	2.0 ± 0.0^{a}	3.0 ± 0.0^{b}	3.0 ± 0.3^{a}	2.0 ± 0.0^{b}	$3.0 \pm {}^{a}$	2.0 ± 0.3^{b}
	1492 - 1527	0.0 ± 0.0^{c}	4.0 ± 0.4^{a}	0.0 ± 0.0^{b}	5.0 ± 0.2^{a}	$0.0 \pm {}^{b}$	5.0 ± 0.1^{a}	0 ± 0^{b}	5.0 ± 0.1^{a}
	F	18.2	8.1	12.7	3.5	11.5	21.4	21.7	41.7
	P	0.003	0.001	0.001	0.05	0.275	0.001	0.001	0.001
	df	15	15	15	15	15	15	15	15
Gubalafto (NW)	1870 - 2044	2.0 ± 0.3^{a}	1.0 ± 0.0^{a}	2.0 ± 0.0^{a}	2.0 ± 0.2^{a}	3.0 ± 0.2^{a}	2.0 ± 0.1^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
	1885 - 1980	3.0 ± 0.1^{a}	2.0 ± 0.1^{a}	3.0 ± 0.4^{a}	2.0 ± 0.1^{a}	2.0 ± 0.4^{a}	2.0 ± 0.2^{a}	2.0 ± 0.1^{a}	1.0 ± 0.0^{a}
	1856 - 1980	2.0 ± 0.1^{a}	2.0 ± 0.2^{a}	2.1 ± 0.1^{a}	2.0 ± 0.3^{a}	2 ± 0.3^{a}	2.0 ± 0.0^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}
	F	0.8	0.9	0.5	1.1	2.3	1.4	0.5	0.2
	p	0.123	0.421	0.421	.234	0.234	0.135	0,132	0.224
	df	11	11	11	11	11	11	11	11
Habru (NW)	1850 - 1889	3.0 ± 0.4^{a}	3.5 ± 1.1^{a}	2.0 ± 0.4^{a}	1.5 ± 0.5^{b}	2.0 ± 0.0^{a}	3.0 ± 0.0^{b}	3.5 ± 0.3^{a}	3.3 ± 0.3^{b}
	1595 - 1670	0.0 ± 0.0^{b}	4.0 ± 0.0^{a}	0.0 ± 0.0^{b}	3.5 ± 0.2^{a}	0.0 ± 0.0^{b}	4.3 ± 0.5^{a}	0.0 ± 0.0^{c}	4.0 ± 0.0^{a}
	1508 - 1670	0.0 ± 0.0^{b}	4.6 ± 0.2^{a}	0.0 ± 0.0^{b}	4.5 ± 0.5^{b}	0.0 ± 0.0^{b}	5.0 ± 0.0^{a}	0.0 ± 0.0^{c}	4.6 ± 0.2^{a}
	F value	54.0	0.65	23.8	37.1	13.4	49.0	139.0	22.9
	P value	0.001	0.53	0.001	0.001	0.001	0.001	0.001	0.001
	df	11	11	11	11	11	11	11	11

Bf = *Busseola fusca*, *Cp* = *Chilo partellus,* mean (± SE) within columns, along each district, followed by the same letters do not differ significantly at the 5% threshold (Student-Newma n-Keuls-SNK).

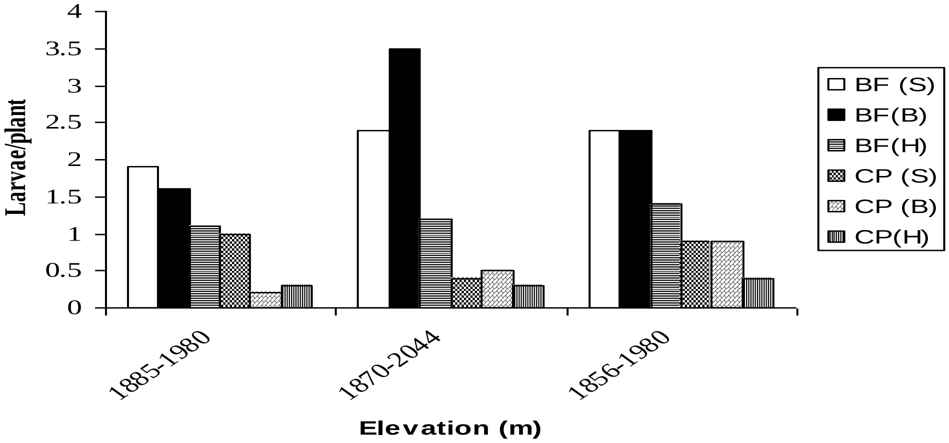


Figure 3. Larval density of *B. fusca* and *C. partellus* at different sorghum growth stages (S = seedling, B = booting, H= harvesting) at Gubalafto district, North Wollo administrative zone in 2010/11.

Table 4. Mean (± SE) stem borer species composition (%) on sorghum and maize in South Wollo and north Wollo zones in 2010/11.

Dist	Altitude (m)	Mean (± SE) composition (%)							
		Maize		Sorghum					
				Seedling		Booting		Harvesting	
		CP	BF	CP	BF	CP	BF	CP	BF
Tehulederi (SW)	2291 - 2338	0.0 ± 0.0^{c}	100 ± 0.0^{a}	0.0 ± 0.0^{c}	100.0 ± 0.0^{a}	0.0 ± 0.0^{c}	100.0 ± 0.0^{a}	0.0 ± 0.0^{c}	100.0 ± 0.0^{a}
	1960 - 1970	11.2 ± 6.5^{c}	88.8 ± 6.5^{a}	29.6 ± 7.1^{b}	70.4 ± 17.1^{a}	31.2 ± 11.2^{b}	68.8 ± 11.2^{b}	8.2 ± 1.9^{c}	91.8 ± 1.9^{a}
	1887 - 1911	27.7 ± 5.1^{b}	72.3 ± 5.1^{b}	19.8 ± 8.9^{b}	80.2 ± 8.9^{a}	35.4 ± 12.5^{b}	64.6 ± 12.5^{b}	20.7 ± 2.6^{b}	79.3 ± 2.6^{b}
	1680 - 1750	100 ± 0.0^{a}	0.0 ± 0.0^{c}	100.0 ± 0.0^{a}	0.0 ± 0.0^{b}	100.0 ± 0.0^{a}	0.0 ± 0.0^{c}	83.6 ± 1.3^{a}	16.4 ± 1.3^{c}
	F	120.0	120.0	20.4	20.4	25.0	38.0	548.7	548.7
	P	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000
	df	15	15	15	15	15	15	15	15
Kalu (SW)	1923 - 2084	7.1 ± 7.1^{b}	92.9 ± 7.1^{a}	27.9 ± 6.6^{b}	72.1 ± 6.6^{a}	41.8 ± 2.1^{b}	58.2 ± 2.1^{b}	42.5 ± 7.8^{b}	57.5 ± 7.8^{b}
	1841 - 1857	9.7 ± 5.7^{b}	90.3 ± 5.7^{a}	27.5 ± 5.2^{b}	72.5 ± 5.2^{a}	34.4 ± 2.9^{b}	65.6 ± 2.9^{a}	41.8 ± 7.1^{b}	58.2 ± 7.1^{b}
	1834 - 1842	19.6 ± 5.5^{b}	80.4 ± 5.5^{a}	33.0 ± 3.4^{b}	67.0 ± 3.4^{a}	31.0 ± 2.5^{b}	69.0 ± 2.5^{a}	24.2 ± 6.7^{b}	75.8 ± 6.7^{b}
	1492 - 1527	100 ± 0.0^{a}	0.0 ± 0.0^{b}	100 ± 0.0^{a}	0.0 ± 0.0^{b}	1000^{a}	0 ± 0.0^{c}	100 ± 0.0^{a}	0.0 ± 0^{a}
	F	70.2	68.9	15.9	15.9	9.2	9.2	27.9	27.9
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	df	15	15	15	15	15	15	15	15
Gubalafto (NW)	1870 - 2044	17.2 ± 7.9^{b}	82.8 ± 7.9^{a}	12.5 ± 4.9^{a}	87.5 ± 4.9^{a}	12.3 ± 2.1^{a}	87.7 ± 2.1^{a}	12.0 ± 2.7^{b}	88.0 ± 5.7^{a}
	1885 - 1980	21.5 ± 4.4^{a}	78.5 ± 4.4^{a}	26.7 ± 9.9^{a}	73.3 ± 9.9^{a}	17.2 ± 2.8^{a}	82.8 ± 2.8^{a}	30.9 ± 6.0^{a}	69.1 ± 6.0^{a}
	1856 - 1980	26.4 ± 12.2^{a}	73.6 ± 12.1^{a}	19.7 ± 13.1^{a}	80.3 ± 13.1^{a}	20.0 ± 5.7^{a}	80.0 ± 5.7^{a}	22.8 ± 1.4^{a}	77.2 ± 1.4^{a}
	F	0.274	0.274	0.509	0.509	0.814	0.814	1.22	1.22
	p	0.767	0.767	0.617	0.617	0.473	0.473	0.0 31	0.339
	df	12	12	12	12	12	12	12	12
Habru (NW)	1850 - 1889	27.4 ± 7.4^{b}	72.6 ± 7.4^{a}	33.4 ± 2.0^{b}	66.6 ± 2.0^{a}	42.0 ± 2.6^{b}	58.0 ± 2.6^{a}	49.1 ± 5.3^{b}	50.9 ± 5.3^{a}
	1595 - 1670	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}
	1508 - 1670	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}
	F	96.2	96.2	1169.9	1169.9	264.2	264.2	42.9	42.9
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	df	12	12	12	12	12	12	12	12

Bf = *Busseola fusca*, *Cp* = *Chilo partellus,* mean (± SE) within columns, along each district, followed by the same letters do not differ significantly at the 5% threshold (Student-Newma n-Keuls-SNK).

contrast, percent damage and number of larvae of *C. partellus* had significant high inverse relationship with elevation with r = -0.92, P < 0.001 and r = -0.83, P<0.001, respectively. Similarly, percent damage and number of larvae of *B. fusca* and *C. partellus* were positively correlated with r = 0.58, P = 0.019 and r = 0.83, P < 0.001, respectively. In addition, temperature and composition of *C. partellus* and *B. fusca* had positive and significant negative correlations with r = 0.83, P = 0.170 and r = -0.91, P = 0.020, respectively.

North Wollo

In Gubalafto district, there was a significant difference between elevations in *B. fusca* damage on maize and sorghum (harvesting). *B. fusca* damage of 23-53 (maize), 20 to 27% (seedling), 21 to 38% (booting) and 5 to 18% (harvesting) of sorghum were recorded at elevations between 1856-2044 m. In the same elevations, *C. partellus* caused damage by 3 to 12% (maize), 4 to 10% (seedling), 11 to 26% (booting) and 2 to 9% (harvesting)sorghum (Table 2). Higher numbers of *B. fusca* larvae (3-4 larvae/plant) were recorded at booting than at seedling stage of sorghum at elevation of >1870 m (Figures 4 and 5).

In this zone, *B. fusca* and *C. partellus* shared 83-88 and 12 to 17%, respectively in elevations between 1856-2044 m.a.s.l. In contrast, low annual rainfall (997-1396 mm) and high temperature (28.8-29.1°C) were recorded at elevations <1670 m.a.s.l. where *C. partellus* shared 100% of the lepidopteran stem borer species complex in elevations < 1670 m.a.s.l. (Table 3).

Moreover, composition of *B. fusca* had a positive relationship with elevation and rainfall (r = 0.99, P = 0.003 and r = 0.79, P = 0. 0.062, respectively) and significant

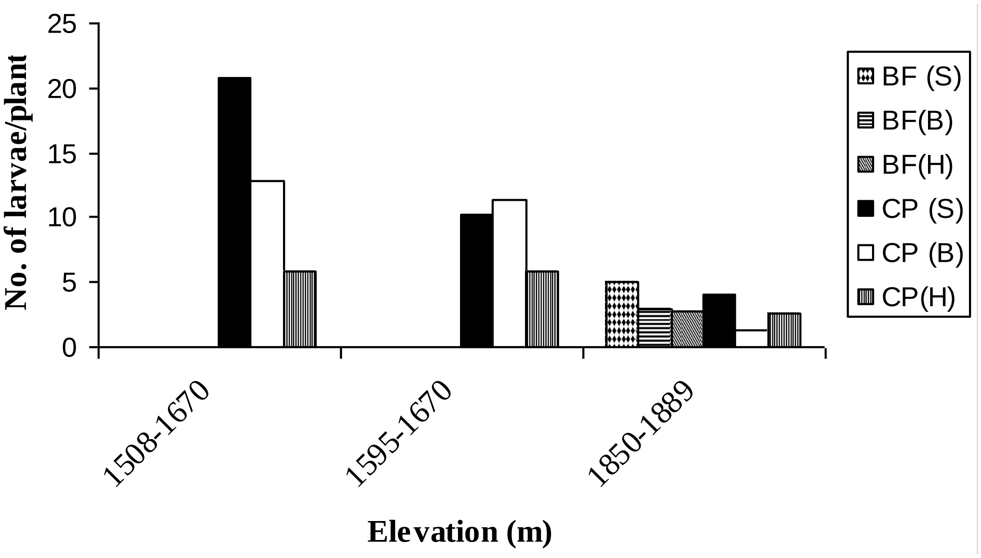


Figure 4. Larval density of *B. fusca* and *C. partellus* at different sorghum growth stages (S = seedling, B = booting, H= harvesting) at Habru district, North Wollo administrative zone in 2010/11.

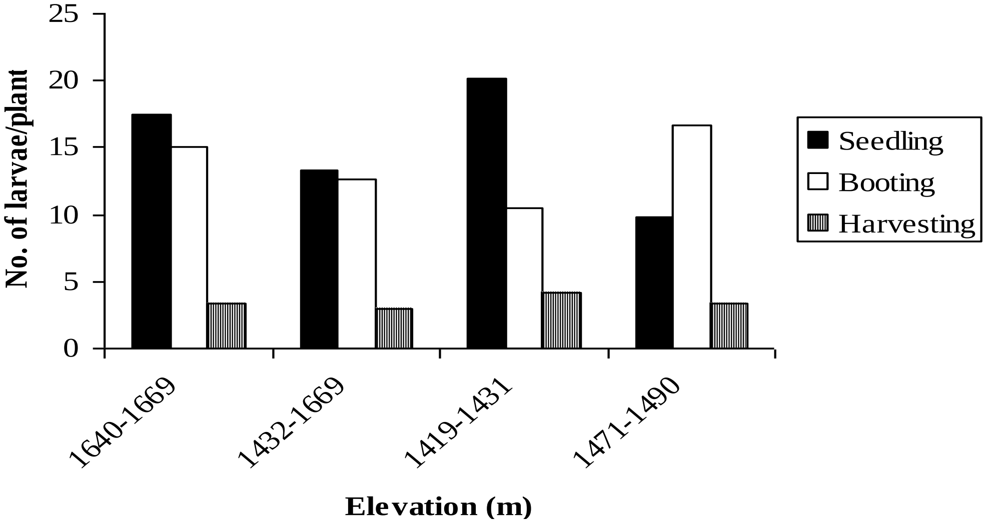


Figure 5. Larval density of *C. partellus* at different sorghum growth stages in Dawa Chefa district, Oromia zone of Amhara region in 2010/11.

inverse relationship with temperature (r = -0.85, P = 0.034). However, composition of *C. partellus* had inverse relations with elevation and rainfall (r = -0.97, P = 0.001 and r = -0.73, P = 0.10, respectively) but a significant positive relationship with temperature (r = 0.87, P = 0.026).

In Habru district, *C. partellus* was the dominant species sharing 100% in elevations <1670 and caused an infestation of 69-94 (maize), 76-98 (seedling), 95 (booting) and 91-92% (harvesting) in the different stages of sorghum. However, it cased significantly lower damage in elevations between 1850 and 1889 m and causing damage of 14 (maize), 4.5 (seedling), 21 (booting) and 15% (harvesting) in the different stages of sorghum (Table 2). The dominant species sharing 51-73% (Table 3) was *B. fusca* causing damage of 37-75% (Table 2). Elevation had significantly high positive correlation with percent damage and the number of larvae of *B. fusca* (r = 0.88, P < 0.0001 and r = 0.86, P < 0.0001) but it had significant high inverse correlation with percent damage and number of the larvae of *C. partellus* (r = -0.98, κ). P<0.0001 and r = -0.88, P = 0.0005). Similarly, percent

Table 5. Mean (±SE) infestation (%) and damage score (1-5 scale) of the two stem borer species on sorghum and maize in Oromia zone in 2010/11.

District	Eleva	Mean (SE ±) infestation (%)				Damage score (1-5 scale			
		Maize	Sorghum			Maize	Sorghum		
			Seedling	Booting	Harvesting		Seedling	Booting	Harvesting
Dawa Chefa	1640 - 1669	92.9 ± 2.4^{a}	89.9 ± 4.6^{a}	91.9 ± 5.2^{a}	86.5 ± 5.6^{a}	4.8 ± 0.1^{ab}	5 ± 0.0^{a}	5 ± 0.0^{a}	4.3 ± 0.3^{a}
	1432 - 1669	84.0 ± 3.2^{a}	90.2 ± 4.1^{a}	94.4 ± 1.3^{a}	91.1 ± 1.0^{a}	4.2 ± 0.3^{b}	5 ± 0.0^{a}	5 ± 0.0^{a}	4.5 ± 0.6^{a}
	1471 - 1490	87.5 ± 4.3^{a}	92.6 ± 4.2^{a}	85.3 ± 6.9^{a}	89.1 ± 2.1^{a}	4.1 ± 0.0^{b}	4.8 ± 0.4^{a}	5 ± 0.0^{a}	4.0 ± 0.4^{a}
	1419 - 1431	93.3 ± 2.7^{a}	99.4 ± 0.6^{a}	94.6 ± 2.5^{a}	95.4 ± 2.0^{a}	5.0 ± 0.0^{a}	5 ± 0.0^{a}	5 ± 0.0^{a}	4.8 ± 0.3^{a}
	F	3.6	1.4	0.91	1.4	5.3	0. 7	1.69	1.1
	P	0.07	0.29	0.465	0.293	0.031	0.421	0.222	0.383
	df	15	15	15	15	15	15	15	15
Bati	1640 - 1657	92.6 ± 1.5^{a}	97.4 ± 1.0^{a}	99.7 ± 0.4^{a}	91.1 ± 2.9^{a}	4.8 ± 0.5^{a}	5 ± 0^{a}	5.0 ± 0.0^{a}	4.3 ± 0.3^{a}
	1555 - 1576	92.8 ± 1.1^{a}	92.2 ± 2.3^{a}	96.5 ± 2.2^{a}	81.9 ± 6.1^{a}	4.5 ± 0.6^{a}	5 ± 0^{a}	5.0 ± 0.0^{a}	3.8 ± 0.5^{a}
	1412 - 1515	90.8 ± 1.9^{a}	96.9 ± 2.4^{a}	98.1 ± 0.8^{a}	88.3 ± 4.4^{a}	5 ± 0.0^{a}	5 ± 0^{a}	5.0 ± 0.0^{a}	4.0 ± 0.0^{a}
	F	0.53	2.14	2.14	1.0	1.3	0.00	0.00	0.6
	P	0.60	0.174	0.174	0.405	0.32	0.34	0.34	0.548
	df	11	11	11	11	11	11	11	11

Bf = Busseola fusca, *Cp = Chilo partellus,* Mean (± SE) within columns, along each district, followed by the same letters do not differ significantly at the 5% threshold (Student-Newma n-Keuls-SN

damage and number of larvae of the two species, *B*. *fusca* (r = 0.85, P<0.0001) and *C. partellus* (r = 0.88, P<0.0001) were positively correlated. In this zone, *B. fusca* caused higher damages than *C. partellus* on the two crops at Gubalafto district but *C. partellus* caused higher damage than *B. fusca* in Habru district except one elevated locality (Figure 7).

Oromia nation zone

The only stem borer species recorded in Dawachefa district were *C. partellus* and shared 100% of the species complex. It caused significantly high damages (84-99%) and high severity scores >4 (>80%) on both maize and sorghum (Table 5). High number of *C. partellus* larval density ranging 10-20 larvae/plant (seedling), 11-17 larvae/plant (booting) and <5 larvae/plant (harvesting) in the different stages of sorghum were recorded in the elevations of 1419-1669 m.a.s.l. (Figure 6).

In Bati district, this species shared 100% of the species complex and caused high damage of maize (91-93%) and sorghum (82 to 100%) with a severity scale of >4 (>80%) within elevations of 1412-1657 m (Table 5). High numbers of larvae per plant were recorded on sorghum at seedling stage (21 - 23 larvae/plant) and maize (10-12 larvae/plant) (Figure 7).

In this zone, *C. partellus* was the most economically important insect that caused severe damage on maize and sorghum.

DISCUSSION

Composition, damage levels and densities of *B. fusca* and *C. partellus* on maize at tasseling and sorghum at different growth stages were varied between elevations of different districts of the three administration zones. In all zones, longer maturing sorghum growth stages were synchro-nized with different rainfall periods, the seedling stage in short rainfall period and the booting stage in main rainfall period.

In Tehulederi district, larval density of *B. fusca* was varied between sorghum growth stages and the highest density was at booting and the lowest at seedling stage. The reason could be that the first generation of this insect occurred in late April after a long larva diapause period during short

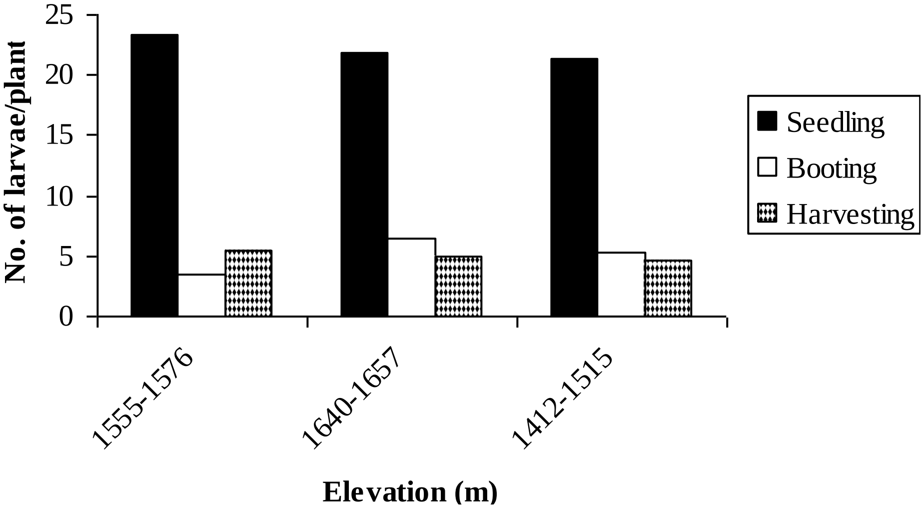


Figure 6. Larval density of *C. partellus* at different sorghum growth stages in Bati district, Oromia zone of Amhara region in 2010/11.

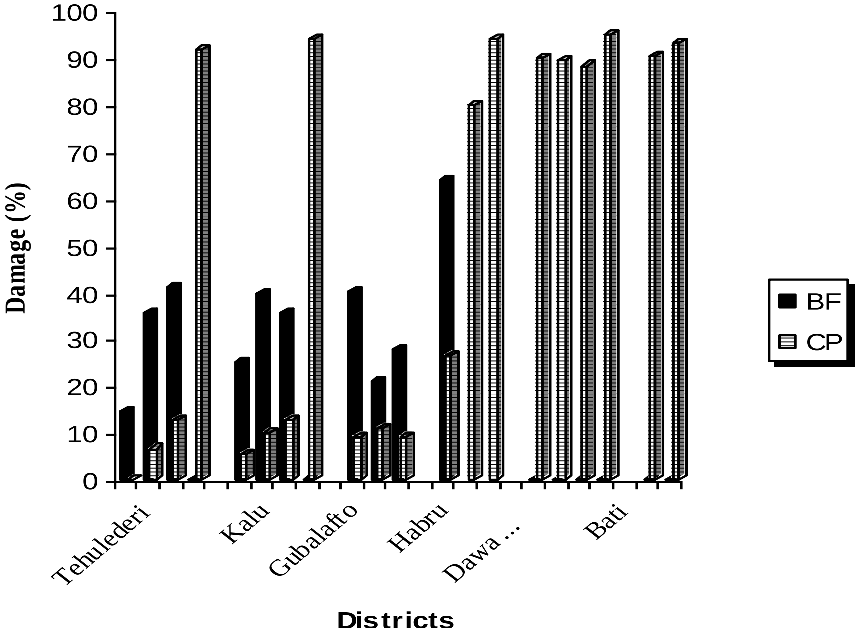


Figure 7. Damage (%) of *B. fusca* and *C. partellus* across each district of the three zones in 2010/11.

rainfall. The long larval diapause period influenced the number of eggs laid and the survival of neonate larvae which depend on the food reservoir of diapause larvae and the second reasons could be the abiotic and biotic factors which reduce the population at each pest stage. In contrast, the second generation occurred in early July (main rain period) after a short larval diapause period that caused a high number of eggs and high survival of neonate larvae. Hence, higher numbers of larvae were recorded at booting stage than at the seedling stage of sorghum. The results also concurred with Kioko et al. (1995). However, because of short larval diapause period and greater than two generations during short rainy period, higher *C. partellus* densities were recorded at seedling than at booting growth stages. Similar results were reported by Bernays et al. (1983), Ampofo and Kidiavai (1987), Kumar (1992),

Kumar et al. (1993) and Odindo et al. (1992). With only one cropping season a year, followed by the long and dry off-season, C. *partellus* should not become a serious problem.

The composition of *B. fusca* varied from 51 to 73% in Habru district (1850-1889 m), 69-88% at Gubalafto (1856-2004 m), 65 to 100% at Tehulederi (1887-2338 m) and 58-93% at Kalu (1834-2084 m) with mean minimum and maximum temperatures of 9.6-13.7 and 19.5-26°C, respectively. This species was not recorded at elevations less than 1770 m, but it shared 16% and caused 2.5% sorghum damage in the 1770 m at Jari with maximum temperature of 27.2°C. *C. partellus* was recorded in all localities where they are situated at elevations between 1492-2084 m. The proportion of *C. partellus* increased from 7 to 100% following the drop of elevation from 2084 to 1492 m and with the increment of temperature from 24.6 to >28.3°C. The result of this study shows that distribution of *B. fusca* had a positive relationship with elevation and rainfall, and an inverse relationship with temperature.

However, distribution of *C. partellus* had inverse relations with elevation and rainfall but a significant positive relationship with temperature. Although, the rainfall, temperature, relative humidity and topographic features are different in different countries of Africa, these results partly in agreement with different authors that *B. fusca* occurs in West Africa from sea level to altitudes in excess of 2000 m but is most abundant in the wetter parts of the tree savannah in Burkina Faso (Nwanze, 1988). In eastern Africa, it occurs between 600 and 2700 m and the species is unable to tolerate mean temperatures of above 27°C (Harris and Nwanze, 1992). Sithole (1989) reported that *B. fusca* is the dominant stem borer at elevations above 900 m and indicating the ability of this pest to adapt to low-lying and the warmer area of South Africa. Tsedeke et al. (2000) also reported that the distributions of *B. fusca* and *C. partellus* are affected by rainfall, temperature and elevation in sub Saharan countries.

In Ethiopia, Emana (2001) reported that *C. partellus* was recorded in elevation between 1030 to 1900 m. In Pakistan, Muhammad et al. (2010) reported that the highest infestation of *C. partellus* was found at a temperature of 32.5°C, low annual rainfall (<1000 mm) and relative humidity of 68%. In Eritrea, it was reported that *B. fusca* and *C. partellus* were important in highlands between 1450-2350 m and low lands less than 1400 m, respectively (Adugna and Hofsvang, 2001). But in contrast to other authors, this result confirmed that *B. fusca* was not recorded in elevation less than 1750 and *C. partellus* was extending its distribution from 1400 to 2084 m. That means it is not only the elevation that influence the distribution of the two species but also other factors like rainfall, temperature, relative humidity and topographic features plays a great role (Nwanze, 1988;). The elevation at which we recorded *C. partellus* is higher than the previous record in Africa which was between 1700-1900 m (Seshu, 1983) and less than 1700 m Assefa (1985).

This result showed that *C. partellus* expanded its ecological niche from 1700 m to >2000 m. Moreover, these survey results show a discrepancy from a previous survey report of the Sirinka Agriculture Research Center (SARC) in 1995 that revealed *B. fusca* was the only lepidopterous species attacking sorghum and maize at Sirinka PA and Gubalafto district with an elevation of 1850-1889 and 1808-2044 m, respectively (Adane and Tesfaye (1998), personal communication).

However, after 15 years of SARC report, this comprehensive survey was conducted and confirmed that *C. partellus* shared 12 to 49% of the stem borer population in these areas and expanded its ecological niche up to 2044 m. This showed that this invasive exotic species co-exists with the native indigenous species, *B. fusca,* and may totally replace it in the near future. Similar to the findings of this research, different authors also reported that *C. partellus* could co-exists in many areas with *B. fusca* and expand its ecological niche from lowlands to highlands (Polaszek, 1998). Kfir (1997) reported that *B. fusca* was the only stem borer found in elevation 1,600 m in South Africa but after *C. partellus* invaded the region, it reached 32% of the total borer population of maize and 59% of sorghum within 5-7 years. The same author reported that *C. partellus* has proven to be an efficient colonizer, and it seems to be displacing the indigenous *B. fusca.* The only species that accounted for 100% of the two stem borer population in elevation less than 1700 m was *C. partellus.* Regardless of crop types and stages, sorghum damage of 70 to 96% and maize damage of 70 to 91% by *C. partellus* was recorded at elevations between 1419-1670 m. Similarly, Gupta et al. (2010) reported that damage magnitude of *C. partellus* ranges from 26.7 to 80.4% in altitude below 1500 m in India. However, damage level of *C. partellus* was as low as 5-21% in elevation between 1850-2044 m. In line with these results, various reports indicated that *C. partellus* distribution is highly influenced by altitude and moisture gradients. In Kenya, for example, *C. partellus* populations are most common in the dry mid altitude and dry coastal areas, but the pest also occurs in the moist-transitional and moist mid-altitude (<1500 m) agro-ecological zones (De Groote et al., 2003).

Our study indicates that the distribution of the two stemborer species are limited by elevations and the data also suggests a trend for increased infestation from high potential to low potential localities, namely the highlands (low infestation) and the lowlands (high infestation). Results from the presented studies provide an increased understanding on the ecology of these stemborer species which have meaningful positive impacts in the population dynamics and in designing effective, economical and safe integrated control measures especially host specific biological control agent like *Cotesia flavipes*, the one parasitizing *C. partellus* is not likely the parasite of *B.*

fusca. Moreover, the information is useful in monitoring and forecasting the two species.

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Full Length Research Paper

Seasonal variation of *Ace-1*^{R} mutation in *Anopheles gambiae s. l.* populations from Atacora region in Benin, West Africa

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The spread of *Ace-1*^{R} mutation that confers carbamate and organophosphate resistance in *Anopheles gambiae s.l.* is a critical issue for malaria vector control. The aim of this study was to investigate the seasonal variation of the *Ace-1*^{R} mutation in *A. gambiae s. l* populations. The mosquitoes analyzed were collected by human landing catches (HLC) in five districts in the department of Atacora from May 2011 to June 2013. The collection was carried out twice a month by adult volunteers. They caught any mosquitoes that landed on their legs with an aspirator from 7.00 p.m to 6.00 a.m. *Anopheles* mosquitoes were morphologically identified and polymerase chain reaction (PCR) assays were run to determine the members of the *A. gambiae* complex, as well as phenotypes for insensitive acetylcholinesterase (AChE1) due to the *Ace-1*^{R} mutation. This study shows that *A. gambiae s. s.* and *Anopheles coluzzii* occurred in sympatry in the Atacora region. However, *A. gambiae s. s.* was predominant during the rainy season, while *A. coluzzii* was predominant during the dry season. Moreover, the *Ace-1*^{R} mutation was detected either in *A. gambiae s. s.* or in *A. coluzzii.* Results showed a dramatical increase in *Ace-1*^{R} allelic frequency during the dry season. The present study provides useful information on the seasonal variation of *Ace-1*^{R} allelic frequency in *A. gambiae* populations from Atacora. It showed that *Ace-1*^{R} allelic frequency has increased in rainy season which can be explained by the various selective insecticide pressures on *A. gambiae* during this season that consist of agricultural practices and indoor residual spraying using bendiocarb.

Key words: *Anopheles gambiae Ace-1*^{R}*,* resistance, seasonal, Atacora, Benin.

INTRODUCTION

Malaria is a major public health problem and *Anopheles gambiae* is one of the major vectors of this disease in sub-Saharan Africa (Gillies and Coetzee, 1987). Malaria vector control mainly relies on the use of insecticide-treated nets (ITN) and indoor residual spraying (IRS) (Beier et al., 2008). Pyrethroids are the only group of insecticides currently recommended for net treatment, the others (organochlorine, carbamate and organophosphate) are applied for IRS (Guillet et al., 1997; Devine and Ogusuku, 2009). The main problem with ITNs and IRS is the development of insecticide resistance, particularly pyrethroid-resistance by several populations of *A. gambiae* (Elissa et al., 1993; Akogbéto and Yakoubou, 1999; Chandre et al., 2000; Akogbeto et al., 2006). The evolution of pyrethroid resistance in *A. gambiae s. s.* represents a threat for malaria control. To prevent any significant decline of the efficiency of pyrethroids, harmful to the malaria control, management of the exploration of new tools or combination

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of existing ones is important.

One of these strategies, used in agriculture as well as in public health, is to use several molecules having different modes of action in the same treatment. Although developed initially for agricultural use and for IRS, carbamates and organophosphates constitute a new prospect to circumvent pyrethroid resistance in *A. gambiae s.s.* For this reason, Benin Republic adopted a national malaria control strategy based on large-scale integrated control measures, which included ITNs and IRS using bendiocarb, a carbamate insecticide.

The department of Atacora has housed a large scale IRS campaign since 2011. However, recent studies reported the increase of bendiocarb resistance in Atacora region and showed the involvement of *Ace-1*^{R} mutation in conferring bendiocarb resistance in *A. gambiae* populations from Atacora (Aïkpon et al., 2013).

Acetylcholinesterase (AChE) is the common target for carbamates and organophosphates. These insecticides block transmission of nerve impulses by irreversible inhibition of AChE at cholinergic synapses, causing insect death. Cross-resistance to carbamates and organo-phosphates can arise by an insensitive AChE mechanism due to the glycine to serine substitution (G119S mutation) resulting from a single point mutation in the *Ace-1* gene (Weill et al., 2003a). The G119S mutation was selected independently in several mosquitoes species including *A. gambiae* s. s., the major malaria vector in Africa (N'guessan et al., 2003; Weill et al., 2003b; Djogbénou et al., 2008).

In the current study, the seasonal variation of *Ace-1*^{R} mutation in *A. gambiae s. l* populations from Atacora was investigated.

METHODOLOGY

Study area

The study was carried out in Atacora, a department located in the north-west of Benin and includes five districts: Kouandé, Matéri, Natitingou, Péhunco and Tanguiéta (Figure 1). The five districts covered 13,778 km^2 and an estimated 482,080 populations in 2012. Atacora region has a sub-equatorial type climate with only one dry season (December-May) and only one rainy season (July-November). The annual mean rainfall is 1,300 mm and the mean monthly temperature varies between 22 and 33°C. The department is irrigated by three major rivers: the Mekrou, Pendjari and Alibori. The major economic activity is agriculture and it is characterized by the production of cotton and millet where various classes of pesticides are used for pest control. Since 2011 onwards the department has conducted a large scale Indoor Residual Spraying (IRS) campaign.

Indoor Residual Spraying (IRS) Campaigns (2011 to 2012)

The product chosen by the NMCP to implement IRS in Atacora was the carbamate class insecticide, bendiocarb (Akogbéto et al., 2010). The formulation was 80% WP. The target dosage was 0.4 g a.i./m^2. Only one round of IRS was carried out per year in the beginning of the rainy season (in June). The IRS operation was performed by volunteers chosen from the local community who were trained by the PMI IRS partner. Each round covered over 90% of the households in the target districts.

Mosquito collection

The mosquitoes analyzed were collected monthly by human landing catches (HLC) in five districts in the department of Atacora from May 2011 to June 2013. For this collection, two villages were selected per district and two houses per village to collect mosquitoes. The collection was carried out twice a month by adult volunteers who have given their informed consent. In each house, a collector was positioned inside and another outside. They caught any mosquitoes that landed on their legs with an aspirator from 7.00 p.m to 6.00 a.m. Considering the risk of malaria transmission, the collectors were given an antimalarial prophylaxis as a prevention against malaria. During the course of this study, all mosquito collectors were monitored for any malaria symptom noticed, an immediate parasitological test took place followed by an antimalarial treatment if necessary.

Laboratory processing of mosquitoes collected

The collected mosquitoes were identified using a morphological identification key of Coluzzi (Co1964). The specimens of *A. gambiae* were stored separately according to the locations they were collected from, then labeled and conserved in Eppendorf tubes containing silica gel. The whole Eppendorf tubes were stored in a freezer at -20°C before any further analyses.

The carcasses (abdomen, legs and wings) of each mosquito were subjected to the *A. gambiae* species specific PCR assays for species identification (Favia et al., 1997). The PCR-RFLP diagnostic test was used to detect the presence of G119S mutation (*Ace.1*^{R} gene). Mosquito genomic DNA was amplified using the primers Ex3AGdir 5′ GATCGTGGACACCGTGTTCG3′ and Ex3AGrev 5′AG GATGGCCCGCTGGAACAG3′ according to Weill et al. (2003). One microlitre of total DNA extracted from a single mosquito was used as a template in a 25 ml PCR reaction containing Taq DNA polymerase buffer, 0.2 mM dNTP and 10 pmol of each primer. The PCR conditions were 94°C for 5 min and then 35 cycles of (94°C for 30 s, 54°C for 30 s and 72°C for 30 s) with a final 5 min extension at 72°C. Fifteen microlitres of PCR product were digested with 5 U of AluI restriction enzyme (Promega) in a final volume of 25 ml. The PCR fragments were fractionated on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Data analysis

Allelic frequencies of G119S mutation were assessed per month and the average was evaluated during the dry season and the rainy season. Allelic frequencies of G119S mutation were analysed using the version 1.2 of Genepop (Raymond and Rousset, 1995).

Ethical consideration

Ethical approval for this study was granted by the Ethical Committee of the Ministry of Health in Benin. The mosquito collectors gave prior informed consent and they were vaccinated against yellow fever. They were also subjected to regular medical check-ups with preventive treatments of malaria.

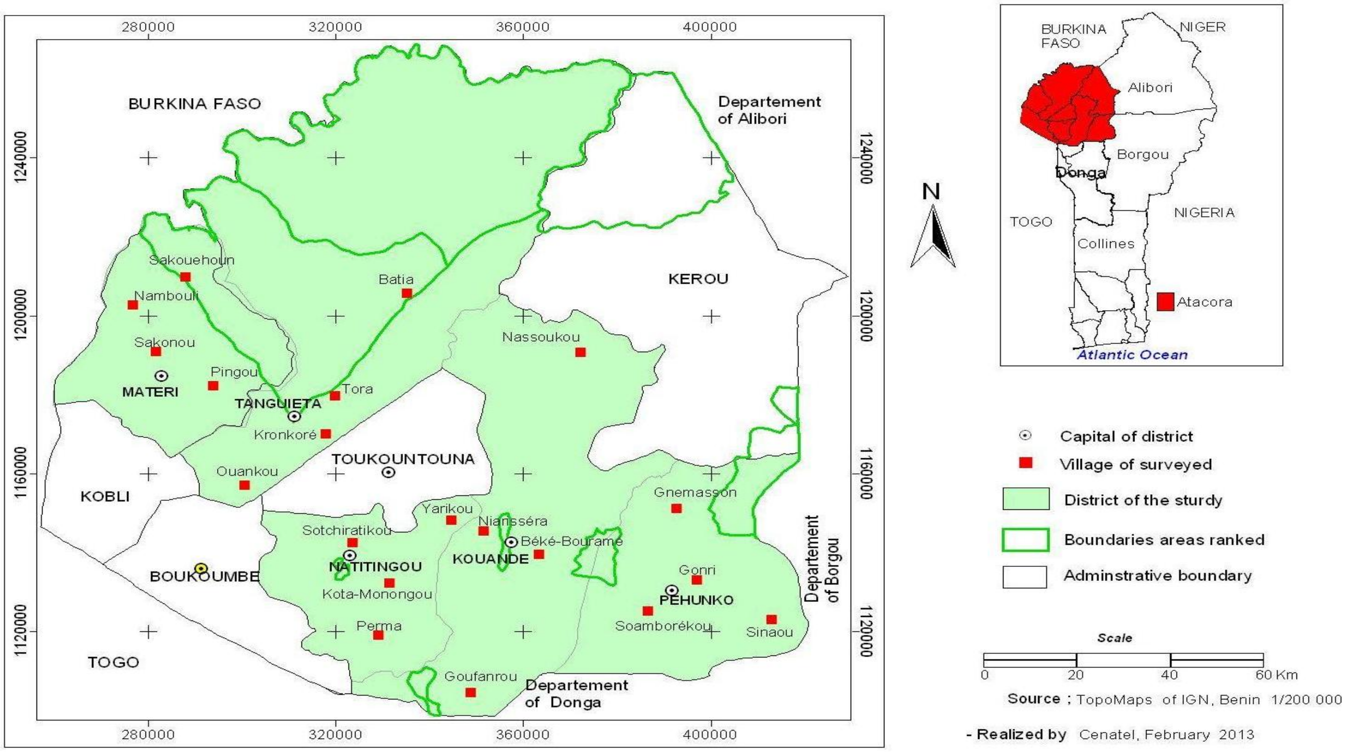

Figure 1. Map of the study area.

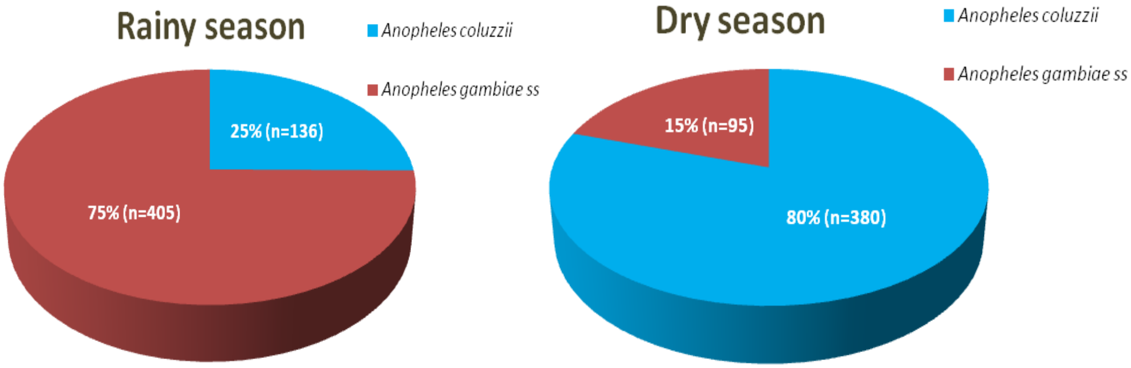

Figure 2. *A gambiae* complex composition and species variation in Atacora region.

RESULTS

A. gambiae s. l species identification

A total of 1016 *A. gambiae* s.l mosquitoes were identified for species identification. Results for *A gambiae* complex composition are shown in Figure 2. *A. gambiae s.s.* and *Anopheles coluzzii* occurred in sympatric in the study area. However, there is an uneven distribution of both species following the dry months and wet months. Indeed *A. gambiae s.s.* was predominant (75%; n = 405) during the rainy season, while *A. coluzzii* was predominant during the dry season (80%; n = 380). Moreover, the *Ace-*1^R mutation was detected either in *A. gambiae s.s.* or in *A. coluzzii.*

Seasonal variation of *Ace-*1^R allelic frequencies

Table 1 presents the *Ace-1* allelic frequency according to the seasons. In all five cities, there was a significant difference between the *Ace-*1^R frequency during the dry

Table 1. Seasonal variation of *Ace-1*^{R} allelic frequency in *A. gambiae* populations from Atacora.

District	Season	n tested	RR	RS	SS	f (*Ace-1*^{R})	OR	CI-95%(OR)	p-value
Kouandé	Dry season	84	0	7	77	04.17^a	0.32	[0.134 - 0.755]	0.0061
	Rainy season	106	4	17	83	12.02^b	1.00	-	-
Matéri	Dry season	56	0	0	56	00.00^a	0.00	[0.000 - 0.481]	0.00097
	Rainy season	140	1	18	121	07.14^b	1.00	-	-
Natitingou	Dry season	118	0	0	118	00.00^a	0.00	[0.000 - 0.123]	<0.0001
	Rainy season	98	2	20	76	12.24^b	1.00	-	-
Pehunco	Dry season	170	0	6	164	01.76^a	0.25	[0.095 - 0.665]	0.0041
	Rainy season	105	0	14	91	06.67^b	1.00	-	-
Tanguiéta	Dry season	47	1	3	43	05.32^a	0.26	[0.100 - 0.709]	0.0036
	Rainy season	92	2	28	62	17.39^b	1.00	-	-

[a] Values sharing the same superscript letter are not significantly different at the 5% level for G119S mutation. OR: Odd ratio; CI: confidence interval. p-value = probability value.

season and the rainy season. This difference is reflected by an increase in frequency during the rainy season. Indeed the frequency is increased from 4.17 to 12.02% of the dry season to the rainy season in Kouandé (p = 0.0061); 0 to 7.14% in Matéri (p = 0.00097); 0 to 12.24% in Natitingou (p < 0.0001); 1.76 t 6.67% in Pehunco (p = 0.0041) and 5.32 to 17.39% in Tanguiéta (p = 0.0036).

DISCUSSION

This study examined target site insensitive acetylcholinesterase (*Ace-1*^{R}) in *A. gambiae* and assessed the seasonal variation of *Ace-1*^{R} allelic frequencies in the Atacora region in Benin.

In this study, two members of *A. gambiae* complex were found in sympatry (*A. gambiae s. s.* and *A. coluzzii*) and their distribution agree with previous findings in Benin that reported both M and S forms with the predominance of S forms in savannah areas (Djogbénou et al., 2010). The presence of *Ace-1*^{R} mutation in *A. gambiae s.s*. and *A. coluzzii* has already been reported by Weill et al. (2004) and Djogbénou et al. (2008), and was suggested to result from introgression between forms.

The low number of homozygous resistant individuals might be related to high fitness cost of the *Ace-1*^{R} mutation, resulting in death of the homozygous resistant mosquitoes (Weill et al., 2004; Asidi et al., 2004; Djogbénou et al., 2010). The high number of heterozygous resistant RS is also in agreement with previous studies which showed that in areas where the resistant allele *Ace-1*^{R} is present, resistant mosquitoes will mainly be in the heterozygote state (RS) (Djogbénou et al., 2010; Ahoua-Alou et al., 2010).

The significant increase in the *Ace-1*^{R} frequency in rainy season can be explained by the use during this season of carbamate in public health that was greatly increased with implementation of IRS. Indeed, the *Ace-1*^{R} frequencies in *A. gambiae* populations have increased and reached high levels in the rainy season after the implementation of IRS campaign. Moreover, agricultural practices using insecticides may also be involved in the increase in *Ace-1*^{R} frequency and resistance to bendiocarb. Indeed, the evidence of an association between agricultural use of insecticides and the emergence of resistance in malaria vectors has been repeatedly reported. For example, in Côte d'Ivoire and Burkina Faso, N'Guessan et al. (2003) reported that the level of vector resistance to pyrethroid insecticides increased during the cotton growing season. Reviewing the impact of agriculture on vector resistance, Mouchet (1998) noted at least 15 malaria vector species for which resistance was directly linked to agricultural treatments. For example, multiple resistance in *Anopheles sacharovi* from southern Turkey has been attributed to heavy usage of organophosphates and carbamates in agriculture (Davidson, 1982). Similar agrochemical selection scenarios have also been described for *Anopheles nigerrimus* in Sri Lanka (Hemingway 1986) and Mexico (Georghiou, 1990). Moreover, pyrethroid resistance in West African *A. gambiae* is thought to have been selected by agricultural treatment, especially those applied to cotton (Penilla, 1998).

Conclusion

The present study provides useful information on the seasonal variation of *Ace-1*^{R} allelic frequency in *A. gambiae* populations from Atacora. It showed that *Ace-1*^{R} allelic frequency has increased in rainy season which can be explained by the considerable pressures, consisting of agricultural practices and IRS, on *A. gambiae* during this season.

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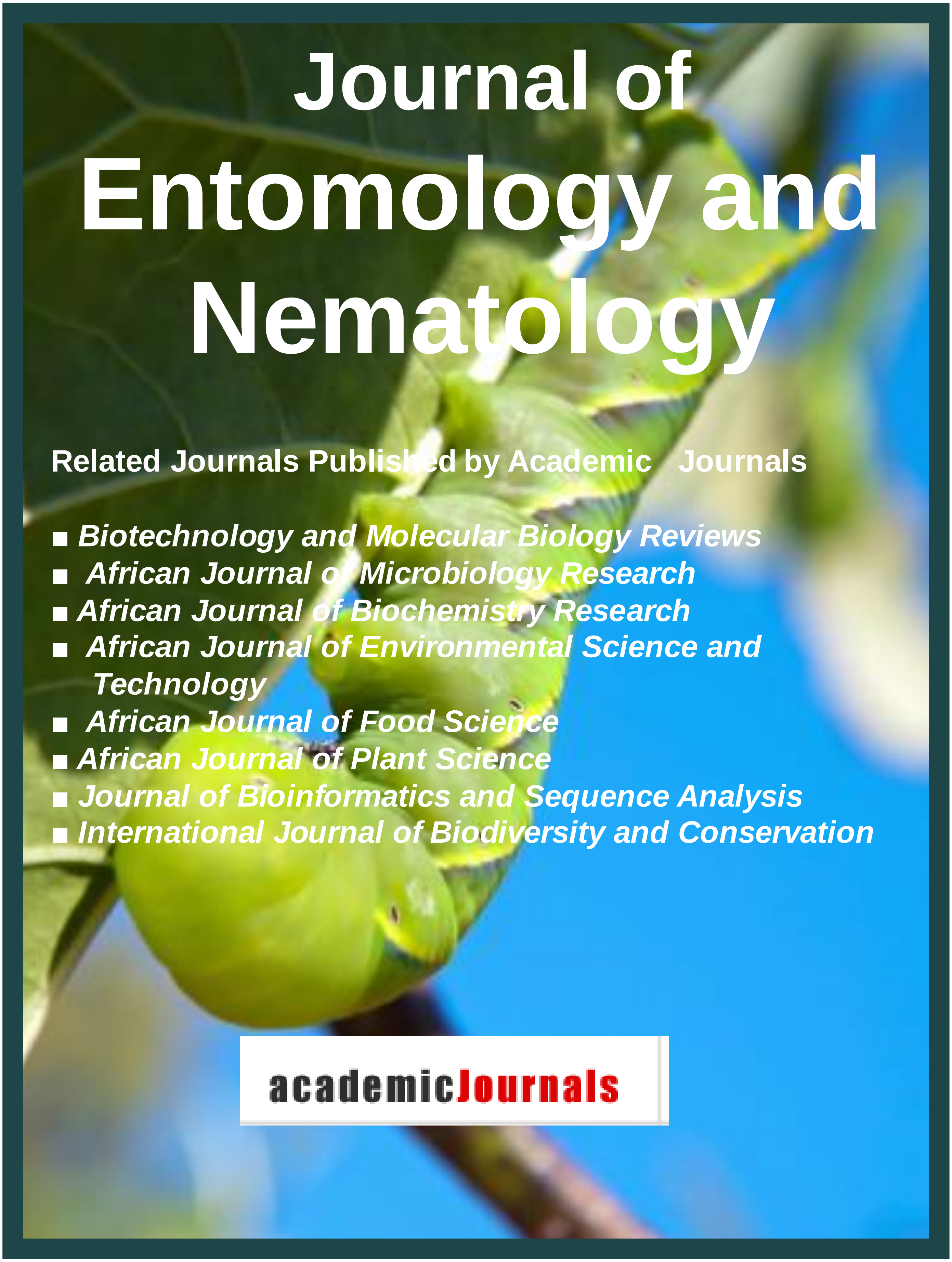

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