ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Tange Denis ACHIRI

SOME BIONOMIC PARAMETERS, MOLECULAR CHARACTERIZATION, AND SUSCEPTIBILITY OF SPOTTED STEM BORER *Chilo partellus* SWINHOE, 1885 (LEPIDOPTERA: CRAMBIDAE) TO SELECTED INSECTICIDES IN ADANA PROVINCE, TURKEY

DEPARTMENT OF PLANT PROTECTION

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ABSTRACT

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SOME BIONOMIC PARAMETERS, MOLECULAR CHARACTERIZATION, AND SUSCEPTIBILITY OF SPOTTED STEM BORER *Chilo partellus* SWINHOE, 1885 (LEPIDOPTERA: CRAMBIDAE) TO SELECTED INSECTICIDES IN ADANA PROVINCE, TURKEY

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In this study, the population dynamics of spotted stem borer, *Chilo partellus* Swinhoe 1885 was investigated in Balcalı, Adana, Turkey in 2018-2019. Also, the susceptibility of blackhead eggs and first instar larvae of *C. partellus* to some insecticides was determined in laboratory conditions. In addition, Turkish populations of *C. partellus* were characterized using molecular techniques. The first observation of *C. partellus* larvae on maize plants was on 03 May and 15 May in 2018 and 2019 respectively. The population of *C. partellus* peaked twice, in May and July in both years. High levels of *Ostrinia nubilalis* Hübner and *Sesamia nonagrioides* Lef. were observed in the second crop of maize for both years. The generalist egg parasitoid *Trichogramm evanenscens* Westwood was recorded on eggs of *C. partellus* for the first time. Deltamethrin reduced hatching of blackhead egg masses of *C. partellus* to 30.62%. All insecticides tested in the study caused mortality of first instar larvae of *C. partellus* greater than 50.0% after 72 h of exposure. Phylogenetic analysis confirmed the identity of the *C. partellus* samples collected by 99-100% nucleotide accuracy. The Turkish populations of *C. partellus* were mixed populations, hypothesized to have originated from India, Kenya and South Africa. This study reveals that insecticides and natural enemies such as *T. evanenscens* mght play a critical role in the management of *C. partellus* in the Mediterranean region of Turkey.

Key words: Adana, *Chilo partellus*, phylogentic analysis, *Trichogramma evanenscens*, Turkey

ÖZ

DOKTORA TEZİ

ADANA İLİ'NDE BENEKLİ MISIRKURDU *Chilo partellus* SWINHOE, 1885 (LEPIDOPTERA: CRAMBIDAE)'UN BAZI BIYONOMIK ÖZELLIKLERI, MOLEKÜLER KARAKTERIZASYONU VE SEÇILI İNSEKTISITLERE DUYARLILIĞI

Tange Denis ACHIRI

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Bu çalışma ile, 2018-2019 yılları arasında Adana (Balcalı)'da benekli mısırkurdu *Chilo partellus* Swinhoe 1885'in popülasyon dinamiği araştırılmıştır. Ayrıca, *C. partellus*'un yumurta (siyahbaş dönemi) ve larvalarının bazı insektisitlere duyarlılığı laboratuvar koşullarında belirlenmiştir. Buna ek olarak, bu zararlının Türkiye popülasyonu moleküler yöntemler kullanılarak tanımlanmıştır. *Chilo partellus*'un birinci mısırda 2018-2019 yıllarında sırasıyla 03 Mayıs ve 15 Mayıs tarihlerinde gözlenmiştir. *Chilo partellus*'un popülasyonu her iki yılda da iki defa tepe noktasına ulaşmıştır. *Ostrinia nubilalis* Hübner and *Sesamia nonagrioides* bireyleri iki yılda da özellikle ikinci ürün mısırlarda yüksek seviyelerde kaydedilmiştir. Genel yumurta parazitoiti, *Trichogramma evanenscens* Westwood ilk kez *C. partellus*'un yumurta parazitoiti olarak kayıt edilmiştir. Deltamethrin *C. partellus* yumurtalarının açılma oranını %30.62 seviyelerine düşürmüştür. Uygulamadan 72 saat sonra, kullanılan tüm insektisitler *C. partellus*'un birinci dönem larvaları üzerinde %50'den fazla ölüme sebep olmuştur. Filogenetik analizler sonucunda örneklenen bireylerin nükleotid dizilimine göre 99-100% oranında *C. partellus* olduğu doğrulanmıştır. Buradan *C. partellus*'un Türkiye popülasyonlarının Hindistan, Kenya ve Güney Afrika'dan gelen karışık popülasyonlar olduğu sonucuna varılmıştır. Türkiye'de Akdeniz Bölgesi'nde insektisitler ve *T. evanenscens* gibi doğal düşmanların bu zararlının mücadelesinde kritik rol oynayacağı düşünülmektedir

Anahtar kelimeler: Adana, *Chilo partellus*, filogenetik analiz, *Trichogramma evanenscens*, Türkiye

EXTENDED SUMMARY

Maize or corn (*Zea mays* L) is an important cereal crop of the family Poaceae, cultivated and consumed almost all over the globe. The constant and steady increase in maize production has been attributed to the wide and growing use of maize and its byproducts. In Turkey, maize is the third inline after wheat and barley among other grains, grown especially in the Mediterranean (33.0%), the Southeastern Anatolia (24.0%), Inner Anatolia (18.0%) and the Aegean region (11.0%) in 2017/2018.

However, in Turkey like many parts of the world, maize production is hindered by many biotic and abiotic factors. Among biotic stresses are lepidopteran (Lepidoptera: Noctuidae, Pyralidae and Crambidae) stem borer pests. There are many lepidopteran pests of maize in Turkey; the major ones include noctuids - *Sesamia nonagroides* Lefebvre and the crambid *Ostrinia nubilalis* Hübner. Both pests caused very severe damage on maize in Turkey especially in the second crop of maize. In 2014, a new lepidopteran pests, spotted stem borer *Chilo partellus* Swinhoe 1885 (Lepidoptera: Crambidae) was recorded for the first time in the Mediterranean regions specifically in Adana, Osmaniye and Hatay Provinces, Turkey. *Chilo partellus* is known to cause very severe damage on maize and sorghum wherever found. *Chilo partellus* is also known to outcompete many native stem borers and exerts itself as the dominant species within a short time. In line with this background, this study was designed to investigate (1) the bionomics of *C. partellus* and population dynamics in relation to other major indigenous maize stem borers and natural enemies in the maize agroecosystem in Adana, Turkey, (2) molecular characterization of *C. partellus* populations in Turkey to determine possible invasive routes and (3) to evaluate susceptibility of *C. partellus* to some commonly used insecticides.

In 2018 and 2019, a maize field was established in the research and implementation area of the Department of Plant Protection, Cukurova University

for the first and second crop of maize from March to October. Three light traps were positioned on the joints forming a triangle around the field. Once a week, 60 maize plants were cut from ground level and taken to the Entomology laboratory where the number of larvae, pupae, egg masses of major stem borers were recorded. Light trap catches were also recorded on the same day. Laboratory reared colonies of *C. partellus* were used for topical and leaf-dip bioassys of commonly used insecticides. In June-July of 2019, *C. partellus* larvae were collected from farmer fields in Hatay, Osmaniye, Mersin and Adana provinces in Turkey. These larvae were maintained in the laboratory and the emerging adults were confirmed as *C. partellus* and used for molecular studies.

In 2018, the first observation of *C. partellus* larvae on the first crop of maize was on 03 May. A major peak population of 6.1 larvae/plant of *C. partellus* was recorded on 10 May. A small (minor) peak of 2.73 larvae/plant of *C. partellus* was observed on 28 June. The first observation of *C. partellus* on the second crop of maize in 2018 was on 05 July. A peak of 5.75 *C. partellus* larvae/plant was observed on 05 July. In 2019, the first *C. partellus* larva on maize plant was observed on 15 May. A major peak population of 0.45 larvae per plant was observed on 28 May on the first crop of maize. In the second crop of maize of 2019, a major peak population of 2.27 larvae of *C. partellus* per plant was recorded on 31 July.

In 2018, the first flight trap catch of *C. partellus* on the first crop of maize was on 26 April. The mean number of *C. partellus* captured and peaked (6.3 adults/trap) on 07 June. For the second crop of maize in 2018, the first flight was observed on 05 July. In 2019, the first flight of *C. partellus* on the first crop of maize was on 03 May. Two peaks were observed; one on the vegetative stage and the other on the dry stage of maize. For the second crop of maize in 2019, the first flight was on 24 July with 2.2 adults/trap. A second small (minor) peak occurred on 18 September 2019.

In 2018, the first sign of stem borer infestation on the first crop of maize was observed on 10 May. The percentage increased from 26.67% (10 May) to 40.0% (24 May). In the second crop of maize in 2018, the percentage increased from 13.3% (5 July) to 96.61% (19 July). In 2019, the first observation of stem borer infestation on the first crop of maize was observed on 15 May at 3.3%. In the second crop of maize of 2019, the percentage rose from 10.16% (24 July) to 76.27% (31 July). After 07 August (66.67%), the percentage infestation remained high above 90.0% through the entire study.

The Taylor aggregation index (b) for the different stages of *C. partellus* at different maize phenology was estimated in order to determine the spatial distribution of *C. partellus*. The value of b in the first crop of maize in 2018 and 2019 were greater than 1 for stages of *C. partellus*. Some b values in the second crop in 2018 and 2019 were below 1.0. In general, the value of b was greater than 1; implying that *C. partellus* shows an aggregated spatial distribution.

The optimum sample units (N) of maize at different population densities of *C. partellus* were estimated according to maize phenology at three precision levels (0.10, 0.20 and 0.30). The higher the precision levels, the higher the number of maize sampling units.

In 2018, the first parasitized egg masses of *C. partellus* were observed on 09 August with a parasitism rate of 71.43%. Parasitism rates reached 100% on 16 August and 11 September. In 2019, the first parasitism of egg masses of *C. partellus* was observed on 31 July at a rate of 20.0%. The highest parasitism rate (50.0%) was observed on 18 September, followed by a parasitism rate of 40.0% on 15 August and 04 September. The parasitoid species of this study was identified as *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on the bases of morphological characters of male genitalia and male antennae.

Two population predictive models (Boltzman type regression and 4-parameter logistic regression) were established using atmospheric degree day

values. Both models predicted the populations of *C. partellus* for 2019 with R^2 values greater than 0.90.

The evaluation of the performance of *T. evanescens* on eggs of *Ephestia kuehniella* reveals that the highest mean number of eggs laid per female of *T. evanenscens* was 43.87 from 25 °C, followed by 41.80 and 28.13 from 20 °C and 30 °C, respectively. The adult female longevity of *T. evanenscens* was 2.53 days from 25 °C followed by 2.47 days and 2.27 days from 20 °C and 30 °C, respectively. The research further reveals that the proportion of females of *T. evanenscens* from all constant temperatures evaluated were significantly greater than those of males.

In the experiment to evaluate the effect of some commonly used insecticides on the blackhead stages of the eggs masses of *C. partellus*, the results indicate that apart from deltamethrin, the other insecticides tested did not influence the hatching of blackhead eggs. The hatching percentages for the insecticides were 98.21%, 91.56%, 30.62%, 89.93%, 95.81%, 89.96%, 86.32%, 95.06% and 97.78% from control, spinosad, deltamethrin, lambda-cyhalothrin, emamectin-benzoate, indoxacarb, chlorpyrifos-ethyl, chlorantraniliprole and novaluron treatments, respectively.

The percentage mortality of first instar larvae of *C. partellus* after 72 hours of exposure to insecticide was 96.67%, 96.30%, 85.55%, 74.08%, 66.67%, 66.67%, 62.59%, 62.22% and 10.37% from emamectin-benzoate, lambda-cyhalothrin, deltamethrin, novaluron, chlorantraniliprole, spinosad, chlorpyrifos-ethyl, indoxacarb and control treatments.

In the experiment to characterize Turkish populations of *C. partellus* using molecular techniques, all the bands in the gel electrophoregram of the cytochrome oxidase subunit I (COI) of the mtDNA were ~710 bp. The gel electrophoregram on the samples from South Africa (Cape Town) was 710 bp. All specimens used in the current study were confirmed as *C. partellus* using mtDNA sequences from the GenBank of NCBI with a 99-100% nucleotide identity. The phylogenetic tree of *C.*

partellus populations in Turkey and South Africa constructed using Maximum Likelihood (ML) with publicly available authenticated barcodes of *C. partellus* in the GenBank of NCBI database, with *O. nubilalis* as an outgroup resulted into three distinct clades after 1000 bootstrap. The ML phylogenetic tree suggests that the Turkish population of *C. partellus* is a mixed population originating from Southern - Eastern Africa (South Africa and Kenya) and from the Indian subcontinent (India).

This study reveals that *C. partellus* infestation of maize in Adana, Turkey start earlier than other major stem borers of maize. This early infestation is a source of damage and yield loss in the first crop. Nevertheless, high parasitism of by the native generalist parasitoid *T. evanescens* in field conditions suggest an important role in the management of *C. partellus*. Therefore, the evaluation of some biological parameters of *T. evanescens* on eggs of *C. partellus* is recommended. High susceptibility of *C. partellus* to many commonly used insecticides in the maize agroecosystem in Turkey was observed in laboratory conditions. Additional experiments are therefore recommended to highlight and underscore the ecological effects of these insecticides in field trials in order to develope IPM strategies. Since the Turkish populations of *C. partellus* is hypothesized to have come from India, South Africa and Kenya, it is recommended that lessons be drawn from these host regions on the control and management of *C. partellus* and adapt to locally available techniques in Turkey.

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SYMBOLS AND ABBREVIATIONS

~	: approximately
±	: plus or minus
° C	: degree celcius
µL	: microlitre
mg	: milligram
kg	: kilogram
ha	: hectare
s	: second
min	: minute
hr	: hour
EB	: Elution buffer
EPPO	: European and Mediterranean Plant Protection Organization
FAOSTAT	: Food and Agricultural Organization of the United Nations statistics
UV	: ultraviolet
ML	: Maximum likelihood
mtDNA	: mitochondrial deoxyribonucleic acid
IGC	: International Grain Council
IPM	: Integrated pest management
PCR	: Polymerase chain reaction
TAE	: Tris-acetate-EDTA buffer
TUİK	: Türkiye İstatistik Kurumu
Turkstat	: Turkish Statistics

1. INTRODUCTION

Maize or corn (*Zea mays* L) is an important cereal crop of the family Poaceae, cultivated and consumed almost all over the globe (Purseglove. 1992; Shah et al. 2016). In fact, it is ranked as the third most important cereal crop after rice and wheat (Sandhu et al. 2007). The production of maize is championed by the United States of America, producing about 361.09 (million metric ton), followed by China (215.812 million metric ton) and Brazil (79.87 million metric ton) in 2014/2015 (FAOSTAT. 2016). This constant and steady increase in maize production has been attributed to the wide and growing use of maize and its byproducts. Traditionally, maize is used as food and feed in various forms (Hawton et al. 1996). However, in the last half of the century, maize has gained wide industrial application (Angel, 2013), for example maize starch is used in paper, textile and pharmaceutical industries (Radosavljevic, 2002; Kurakake et al., 2009; Liu et al., 2015). Maize is also used in the production of biogas (Schwietzke et al., 2009; Tang et al., 2011). According to Erickson (2006), corn oil has become an important item of products manufactured from maize. Corn oil is known to reduce serum cholesterol in humans (Ambika et al., 2012).

In Turkey, maize is the third inline after wheat and barley among other grains, grown especially in the Mediterranean (33.0%), the Southeastern Anatolia (24.0%), Inner Anatolia (18.0%) and the Aegean region (11.0%) in 2017/2018 (TurkStat, 2018). Over the last ten years, maize production has increased steadily both in production area (ha) and production quantity as shown in Figure 1.1 (TurkStat, 2018). It should be noted that although Turkey is ranked as the 8th maize producer of the world coming just behind Russia, the maize yield per hectare (ton/ha) in Turkey is 3rd in the world at about 9.1 ton/ha following Canada (10 ton/ha) and the USA (11.1 ton/ha) in 2017/2018 (IGC, 2018).

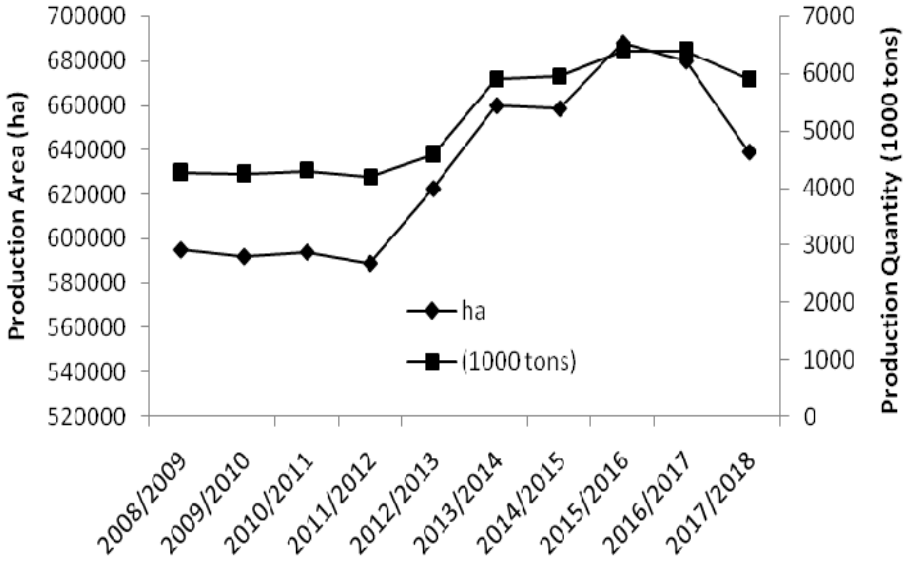


Figure 1.1. Maize production in Turkey in 2008 – 2018 (Turkstat, 2018)

In Turkey like many parts of the world, maize production is hindered by many biotic and abiotic factors. Among biotic stresses are lepidopteran (Lepidoptera: Noctuidae, Pyralidae and Crambidae) stem borer pests. According to Pingali (2001) and James (2003), maize stem borers are a very limiting factor to maize production worldwide, causing about 10 – 70% loss (Kfir et al., 2002: Sarup et al., 1987), and sometimes 100% loss in severe infestations especially in Africa (Overholt et al., 2001). In Sub-Saharan Africa (SSA) the major maize stem borers causing economic damage includes, *Busseola fusca* (Fuller) (Noctuidae), *Sesamia calamistis* (Hampson) (Noctuidae), *Eldana saccharina* (Walker) (Pyralidae), *Chilo orichalcociliellus* (Strand) (Crambidae) and *Chilo partellus* (Swinhoe) (Crambidae) (Kfir et al., 2002; Krüger et al., 2008; Addo-Bediako and Thanguane, 2012).

There are many lepidopteran pests of maize in Turkey including noctuids - *Sesamia nonagroides* Lefebvre, *Spodoptera exigua* Hübner, *Spodoptera littoralis* Boisduval, *Helicoverpa amigera* Hübner, *Pseudaletia unipuncta* Haworth,

Mythimna [*Acantholeucania*] *loreyi* Duponchel and the Crambid *Ostrinia nubilalis* Hübner, and they cause significant crop damage from seedling emergence to harvest (Sertkaya et al. 2004: Sertkaya and Bayram 2005: Bayram et al. 2007). However, *S. nonagrioides* and *O. nubilalis* are the major pest of maize in Turkey; causing about 5.0% in the first crop and up to 100% yield loss in the second crop or late planted corn (Tsitsipis, 1988; Kayapınar and Kornoşor, 1992; Güllü, 2000; Sertkaya and Kornoşor, 2000; Sertkaya et al., 2004; Öztemiz et al., 2011). More recently, in 2014 was added a new lepidopteran spotted stem borer *Chilo partellus* Swinhoe 1885 (Lepidopteran: Crambidae) in the Mediterranean regions specifically in Adana, Osmaniye and Hatay Provinces, Turkey (Sertkaya et al. 2014). *Chilo partellus* is known to cause very severe damage on maize and sorghum wherever found (Kfir et al., 2002; Arabjafari and Jalali, 2007). Not only does it damage the vegetative parts of the plant, but it also damages the reproductive parts, causing damages between 24-75% (Kumar, 2002), and 80-100% in severe infestation in Asia and Africa (Overholt et al., 2000; Arabjafari and Jalali, 2007). The classical damage patterns characteristic of maize stem borers include bored holes on stems and destroyed internal stem tissue resulting into tunnels (Slabbert and Van den Berg, 2009) and a mixture of rotten pungent insect and plant tissue debris within the stems. At the end of the day, the damaged plant is prone to toppling and lodging from any form of perturbation such as heavy rains, storms and strong winds.

In order to effectively manage any pest, it is important to understand the population dynamics of the pest in relation to some abiotic factors (temperature and humidity) and to indigenous pests and natural enemies *in situ*. Thus, the aim of this study is to investigate the bionomics of *C. partellus* and population dynamics in relation to other major indigenous maize stem borers and natural enemies, molecular characterization of *C. partellus* to determine possible invasive routes and to evaluate susceptibility of *C. partellus* to some common pesticides. This

information will provide important database relevant for proper integrated pest management of *C. partellus* and other native maize stem borer pests.
The specific objectives were:

- To ascertain the seasonal prevalence of *Chilo partellus* and characterize interactions and dynamics with other major indigenous lepidopteran stem borers such as *Ostrinia nubilalis* and *Sesamia nonagrioides* in the first and second crops of maize in the agroecosystem of Adana.
- To evaluate some biological characteristics of the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) of *C. partellus* under different constant temperatures.
- To assess the susceptibility of *C. partellus* to some insecticides in laboratory conditions.
- Using molecular techniques to understand the genetic diversity and possible trace the invasive route of *C. partellus* into Turkey.

2. LITERATURE REVIEW

2.1. Taxonomy and description of *Chilo partellus*

2.1.1. Taxonomy

Chilo partellus is a lepidopteran pest belonging to the Family Crambidae. *C. partellus* was formerly in the Family Pyralidae. *C. partellus* was described by Swinhoe in 1885 (Kalaisekar et al., 2017). A common synonym of *C. partellus* is *Chilo zonellus* (EPPO, 2000). In English literature, *C. partellus* is commonly called the spotted stem borer (STB), dura stem borer, sorghum stem borer and spotted stalk borer (EPPO, 2000).

2.1.2. Description of *Chilo partellus*

2.1.2.1. Egg

Chilo partellus usually lays eggs on the upper (dorsal) surface of the leaves closed to the midrib. The eggs are laid in a herringbone fashion (Kalaisekar et al., 2017). The eggs can also be laid in batches mostly on the lower side of the lower leaves on both the ventral and the dorsal surfaces (Figure 2.1) (Pats and Ekbom, 1994). The average number of eggs per batch is 40; however, the number ranges from 10 – 168 especially in overlapping batches (Akol et al., 2011). The egg is about 0.8mm long. The egg colour changes progressively from cream white to pale yellow and eventually black, approximately 2 days or less to hatching a condition called ‘blackhead’ (Kfir, 1993).

2.1.2.2. Larvae

Chilo partellus larvae are creamy-white to yellow-brown in colour (Akol et al., 2011). The larvae posses two longitudinal dorsal series of spots between two dorsolateral series of spots forming four dark spots per segment (Kalaisekar et al., 2017). These dark spots per segment are very conspicuous, giving the larvae a

spotted appearance, thus its name ‘spotted stem borer’ (Akol et al., 2011; Kalaisekar et al., 2017). Each spot has about 3-4 radiating setae in the first instar larvae. Fully grown larvae are dark brown with reddish-brown head (Figure 2.1) (Akol et al., 2011).

2.1.2.3. Pupae

The pupae are obtect measuring about 15 mm long. The pupae are slender, shiny and light yellow-brown to dark red-brown in colour (Akol et al., 2011). The pupae have apical protruberances and indented lines around in segments 5-7 (Kalaisekar et al., 2017) as shown in Figure 2.1.

2.1.2.4. Adults

Adults of spotted stem borer are relatively small to other moths with wing length of 7-17 mm and wingspan of 20 – 25 mm (Figure 2.1). The forewing have darker scale patterns forming longitudinal stripes at the margin (Akol et al., 2011). In males, the hind wings are pale and white in female. The females are larger than the males (Kalaisekar et al., 2017).

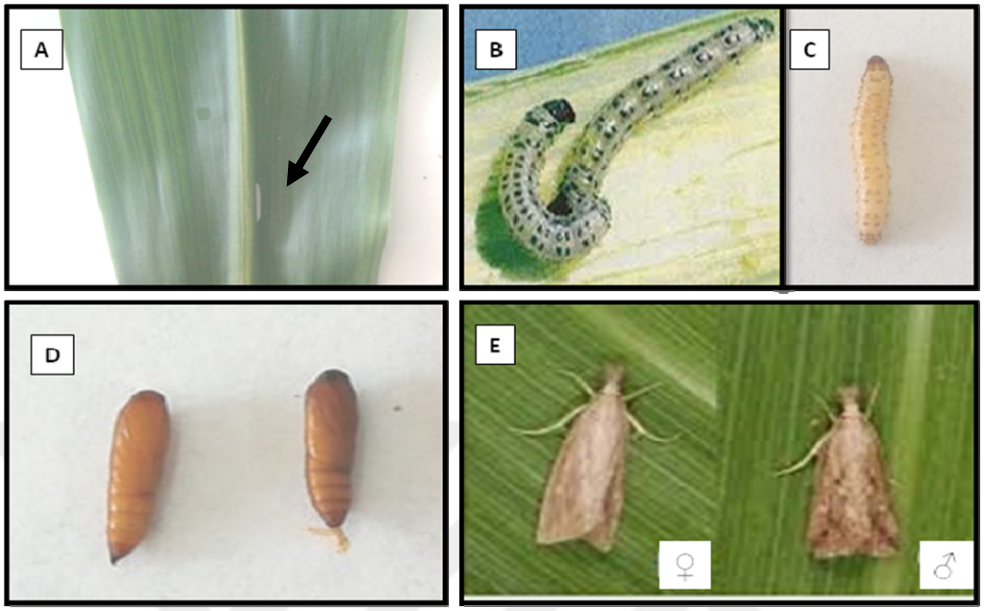


Figure 2.1. Life stages of *Chilo partellus*: A – egg laid closed to the midrib of leaves in a herringbone pattern; B – first to second instar larvae with setae on spot; C – mature larvae (sixth instar) with a reddish-brown head; D – pupae; E – adult. (@ Achiri.

2.2. Origin, distribution, spread and host range of *Chilo partellus*

2.2.1. Origin and distribution of *Chilo partellus*

Chilo partellus is reported to originated from Asia (Harris, 1990) particularly from the Indian Subcontinent (Jalai and Singh, 2003; CABI, 2007). Swinhoe (1885) first recorded *C. partellus* in India. Since then, *C. partellus* has been recorded in various Central, South and East Asian countries including Afghanistan, Bangladesh, Cambodia, India, Indonesia, Iran, Laos, Nepal and Pakistan, Sri Lanka, Thailand, Vietnam and Yemen (Harris, 1990; Yonow et al., 2017). *Chilo partellus* was first reported in Africa in Malawi in 1932 (Tam, 1932). Years later, *C. partellus* has spread to many eastern and southern Sub-Saharan, and a few western countries in Africa including Bostwana, Eritrea, Ethiopia, Kenya, Mozambique, Somalia, Sudan, South Africa, Lesotho, Swaziland, Tanzania,

Uganda, Zambia, Zimbabwe, Cameroon, Togo and the Island, Comoros and Madagascar (Harri, 1990; Kfir et al., 2002; De Groote et al., 2003; Tefera, 2004; CABI, 2007; Yonow et al., 2017). Yonow et al. (2017) has indicated the countries on the globe where *C. partellus* has been recorded (Figure 2.2).

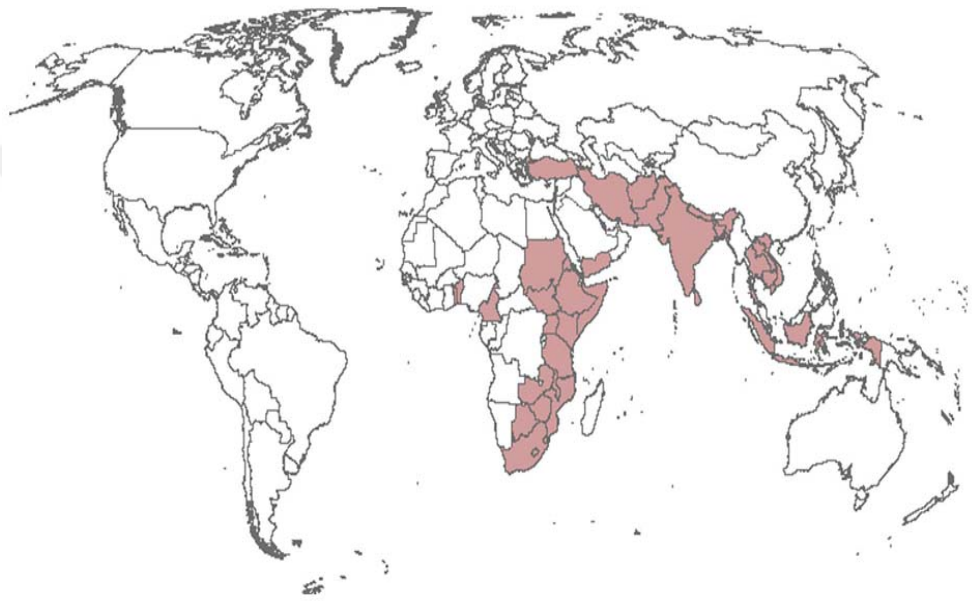


Figure 2.2. Map of the world, with shaded areas showing those countries in which *Chilo partellus* has been recorded (Yonow et al., 2017).

2.2.2. Spread of *Chilo partellus* and displacement of indigenous stem borers

Chilo partellus has been spreading in its range in Africa, particularly from warmer lowlands into higher altitudes (Kfir, 1993; Guofa et al., 2001) and recently in the Mediterranean regions (Yonow et al., 2017). Ben-Yakir et al. (2013) and Sertkaya et al. (2014) has reported *C. partellus* in Israel and Turkey, respectively. This rapid spread of *C. partellus* is enhanced by its ability to displace native species of many injurious stem borers (Seshu, 1983; Kfir, 1997a), and in many cases becoming the major stem borer (Kfir, 1997b; Polaszek, 1998), for example Ofomata et al. (2000) reported evidence that *C. partellus* has partially displaced the

indigenous stem borer *C. orichalcociliellus*. Likewise, Kfir (1997b) reported that *C. partellus* has partially displaced *B. fusca* over a period of seven years in the eastern Highveld region of South Africa.

Several reasons have been suggested why *C. partellus* shows competitive advantage over some indigenous stem borers. Firstly, hibernating larvae of *C. partellus* terminate diapauses and emerge about a month earlier than *C orichacociliellus* and *B. fusca* (Kfir, 1997b; Ofomata et al., 1999; Dejen et al., 2014) which probably allows *C. partellus* to colonize host plant before the native stem borers at the start of the growing season. Secondly, the life cycle of *C. partellus* is about 20 days shorter than that of *B. fusca*, further increasing its competitive advantage (Dejen et al., 2014). Furthermore, it is reported more neonates of *C. partellus* larvae spread to other plants from plants where they hatched, over greater distances than *C. orichalcociliellus*, thus colonizing more plants relative to indigenous stem borers (Ofomata, 1997). Kfir (1997b) suggested that *B. fusca* can discriminate and avoid plants already infested with *C. partellus* using volatiles from host plants feeding.

2.2.3. Host plants

Maize spotted stem borer like many lepidopterous stem borers are polyphagous, feeding on many gramineous and other non cultivated wild host plants (Cugala, 2002). *Chilo partellus* prefers maize and sorghum (Mushore, 2005). They have also been reports of *C. partellus* on Pearl millet, rice, wheat and sugarcane in the field (Mushore, 2005). Chinwada and Overholt (2001) reports that *C. partellus* has been reported on about 24 wild host plants species in the family of Graminae. Ong'amo et al. (2016) has reported the presence of *C. partellus* on wild grasses including, *Sorghum arundinaceum* (Desv.) Stap, *Megathyrsus maximus* (Jacq.) B.K Simon & S.W.L. Jacobs, *Pennisetum purpureum* Schumach, *Arundo donax* L, *Vossia cuspidata* (Roxb.) Griff., *Phragmites mauritianus* Kunth,

Rottboellia cochinensis (Lour.) W.D. Clayton, and *Echinchloa pyramidalis* (Lam.) Hitchc. & Chase.

2.3. Life cycle and seasonal abundance of *Chilo partellus*

2.3.1. Life cycle

The biology of *C. partellus* is very temperature dependent. Successful development of *C. partellus* from egg to adult is between 30–40 days with the conditions of 25-30 ^{o}C and 60-70% Relative Humidity (RH%) (Kfir, 1997b; Ong'amo et al., 2016). Eggs are laid in batches on the leaf surface usually closed to the midrib and the hatch after 4-10 days (Akol et al., 2011). Each female can lay up to 225 eggs (Kfir, 1997b). Young larvae move up into the whorl and feed, later older caterpillars tunnels down into the stems, eating and creating galleries (Ong'amo et al., 2016). The larval stage last about 2–3 weeks, then pupates and remain in the stem. Adults emerge within 7-14 days (Akol et al., 2011; Ong'amo et al., 2016). Majority (75%) of the *C. partellus* males hatch within the first five hours of the scotophase (20.00-08.00h), females hatch five hours later, and this asynchrony of eclosion time fascilitates mating within the last five hours of the scotophase on the night of eclosion (Pats, 1991). Unnithan and Paye (1991) reported that about 80-85% of mating is achieved within 1-2 days of eclosion. Oviposition occurs at the first five hours of the scotophase with about 53.17% of oviposition occurring in the first night (after eclosion), 24.88% the second night and 21.95% the third night (Pats, 1991).

Chilo partellus undergoes aestivation and hibernation in a diapausing conditions of LD 13:11 h photocycle, 32 ± 1 ^{o}C, 65 ± 5% RH and LD 10:14 h photocycle, 10 ± 1 ^{o}C, 65 ± 5% RH, respectively (Dhillon and Hasan, 2018). Although temperature is the key factor inducing diapauses, it is also well documented that the nutritional environment of *C. partellus* can induce diapauses (Delobel, 1975; Scheltes, 1978). For instance, an increase in carbohydrates and decrease in protein and water can initiate diapauses even when climatic conditions

are favourable for development of *C. partellus* (Scheltes, 1978). The life cycle of *C. partellus* is summarized in Figure 2.3.

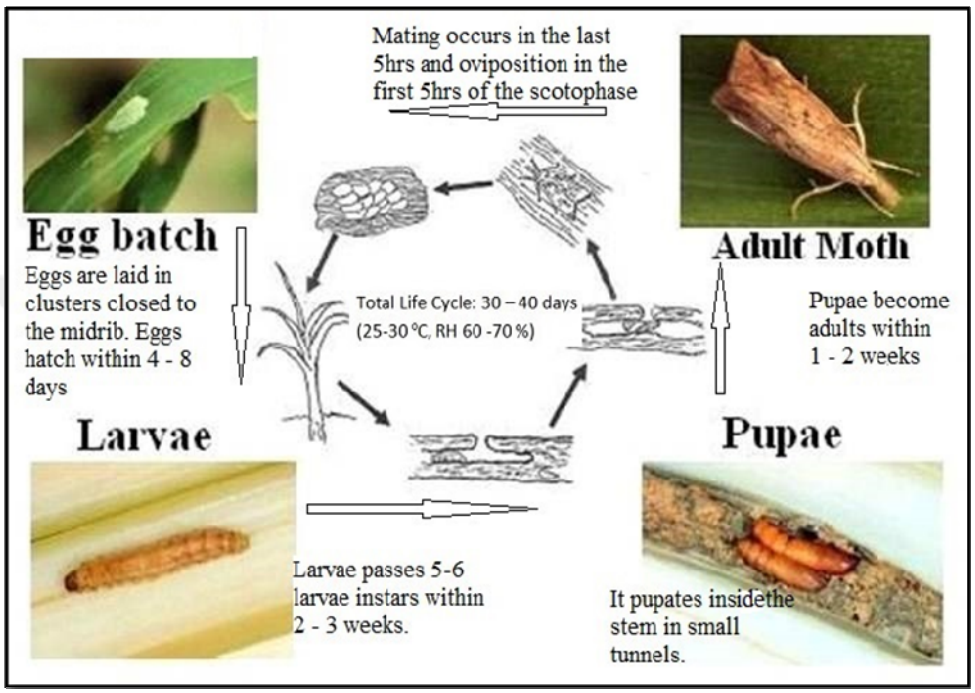


Figure 2.3. Life cycle of *Chilo partellus* (adopted from ICIPE by Hutchison et al. 2008)

2.3.2. Seasonal abundance of *Chilo partellus*

Depending on the location and the number of maize cropping seasons or other host available throughout the year, *C. partellus* completes about 1, 2 or more generations per year (Ong'amo et al., 2016). The seasonal abundance of *C. partellus* like many other maize stem borers in Africa varies differently with many of them occurring as a complex of species with overlapping spatio-temporal distribution (Chabi-Olaye et al., 2001). For example in Kenya where maize is sown for the first growing season between October and April, and May-June for the second growing season, *C. partellus* peaks from about February to June (Kfir, 1997b). In South Africa, *C. partellus* starts to peak in June until March with an

optimal peak in November (Krüger et al., 2008). In India, Singh and Sharma (1984) reported severe attack by *C. partellus* in August and decline in September-October in Punjab. Generally, high infestation rate is recorded between July and September in India (Mohan et al., 1990; Jalai and Singh, 2003). In Turkey, extensive elaborate study on the seasonal abundance of *C. partellus* has not been done. However, one-year preliminary study conducted in Adana, Turkey, indicated that a 2-3 peak of *C. partellus* population was observed with the first peak in May and a second peak in July (Achiri et al., 2018).

2.4. Pest status of *Chilo partellus* and yield losses of maize

Chilo partellus is known to cause very severe damage on maize and sorghum wherever found (Kfir et al., 2002; Arabjafari and Jalali, 2007). Not only does it damage the vegetative parts of the plant, but it also damages the reproductive parts, causing damages between 24-75% (Kumar, 2002), and 80-100% in severe infestation in Asia and Africa (Overholt et al., 2000; Arabjafari and Jalali, 2007). The classical damage patterns characteristic of maize stem borers include bored holes on stems and destroyed internal stem tissue resulting into tunnels (Slabbert and Van den Berg, 2009) and a mixture of rotten, pungent insect and plant tissue debris within the stems. Consequently, the damaged plant is prone to toppling and lodging from any form of disturbance such as heavy rains, storms and strong winds. In addition, *C. partellus* like many other stem borers feed and destroy the growing apex of the maize plant resulting into a condition called 'deadheart'; the growing central leaves die, the plant become stunted or/and eventually dies (Rauf et al., 2017).

2.5. Management of *Chilo partellus*

Several control and management methods to curb the damage by *C. partellus* have been reviewed by Kfir et al. (2002). Murenga et al. (2011) also briefly documented some control strategies for *C. partellus* and their shortcomings.

Pheromone-baited traps have been exploited in an attempt to control *C. partellus*. However, Campion and Nesbitt (1983) concluded that sex pheromones used for mass trapping are unlikely to effectively control *C. partellus* although mating disruption can be effective.

Habitat management practices such as intercropping are also successfully reducing infestation (Ampong-Nyarko et al., 1994; Pats et al., 1997) of up to 30% in maize/sorghum/cowpea-intercropping systems. Other cultural practices such as manipulating planting dates (Van Hamburg, 1979), management of crop residue (Pats et al., 1997), and fertilizer application are also being investigated and used (Van den Berg and Van Ransberg, 1991). Nevertheless, Harris (1989) reported cultural practices are not fully adopted by farmers, especially poor resource farmers due to lack of adequate support and extension services.

Resistant maize varieties have been tried with some success (Ajala et al., 1995; Ahmed et al., 2003; Murenga et al., 2011). Parasitoids such as *Cotesia* spp. and *Trichogramma* spp. have also been used for control of *C. partellus* with relative success in many parts of the world (Kfir et al., 2002; Ahmed et al., 2003). In India, Rai and Prasad (2019) recorded a natural parasitism rate of 57.0% of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) on the larvae of *C. partellus*. In the sub-moist (lowland) northeastern Ethiopia, a parasitism rate of 33.0% and 82.0% of *C. flavipes* on larvae of *C. partellus* was recorded by Dejen et al. (2013). Parasitism rate of 33.0%-80.0% from the egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) has been recorded in many different parts in India (Jalali and Singh, 2006; Kumar and Kanta, 2011; Aziz and Gaherwal, 2017). Can Cengiz et al. (2016) also reported two egg parasitoids; *Telenomus busseolae* Gahan (Hymenoptera: Platygastridae) and *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) on *C. partellus* in Turkey.

Recently push-pull technology, which is a stimulo-deterrent technology, has gained prominence with a huge adoptability rate especially in the eastern parts of Africa (Pickett et al., 2014). In the ‘push-pull’ system, a pest is repelled or

deterred (‘push’) from the main crop and simultaneously attracted (‘pull’) to other areas such as traps or trap crops where they can be easily controlled. With regards to push-pull technology, brachiara and Napier (*Pennisetum purpureum*) grass are amongst the most commonly used ‘pull’ grasses and silverleaf desmodium (*Desmodium uncinatum*) as ‘push’ grass (Van den Berg, 2006; Cheruiyot et al., 2017). Khan et al. (2000) reported that planting napiar grass around a plot of maize resulted into a significant stem borer infestation, with a resulting yield increase of 1 – 1.5 t/ha. Essential oils (Sing et al. 2009) and microorganism-based products (Odindo, 1990; Poinar and Polaszek, 1998) are also gaining grounds.

Insecticides applied in foliar and granular formulations are widely used as preferred solution by farmers because they remain the quickest and fasted control method (Rauf et al., 2017). The insecticides are most effective if applied in the initial stages of an infestation in order to prevent egg hatching and get rid of first and second instar larvae before burrowing into the maize stems (Kfir et al., 2002; Kumar, 2002). In Pakistan, foliar application of fenvalerate, endosulfan, cypermethrin, monocrotophos, quinalphos, and granular applications of chlorpyrifos-ethyl and carbofuran are conventionally recommended for management of maize stem borers (Mathur and Satyadev, 1992; Katole and Mundiwate, 1995; Bhat and Baba, 2007). Entomopathogens such as *Beauvaria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) (Odindo 1991; Maniania, 1993; Gardeze et al., 1998), and novel insecticides such as novaluron, spinosad, emamectin-benzoate have also shown very positive results against *C. partellus* (Rameash et al., 2012). Cypermethrin, carbofuran and methamidophos are commercially available against maize stem borers in India (Khan and Amjad, 2000). According to Van den Berg and Van den Westhuizen (1995), endosulfan and deltamethrin are also used against *C. partellus* in South Africa. There are many concerns about the negative impacts of insecticides use on human health and the environment (Achiri et al., 2016; Guesdes et al., 2016).

Nevertheless, judicious use of insecticides provides a very fast and effective control against many pests.

2.6. Management of invasive species

According to Grice (2009), any program aimed at eradicating, containing or managing invasive species should be based on an evaluation of their impacts. The impact of *C. partellus* damage in Turkey has not been elaborately evaluated. In such circumstance, Grice et al. (2004) proposed that extrapolations from previous research findings gathered elsewhere in corroboration with local observations and informed opinions should be sufficient to give a species' impact in the new environment where extensive studies have not been done. Based on the conclusions of Grice et al. (2004) and knowledgement of economic damage level of *C. partellus* in many parts of the world, it is therefore important for control measure be taken in Turkey.

The first principle in the management of invasive species is to obtain information on their distribution and abundance (Grice, 2009). Sertkaya et al. (2014) reported the distribution of *C. partellus* in the Mediterranean belt of Turkey although no coherent investigation on the seasonal abundance has been done.

Another important principle in the management of invasive species is to determine the ecological history of the species (Donlan and Martin, 2004). One way to determine the origin of an invasive species is to examine its genetics in order to trace their locus of origin and learn their natural and aided control (Baskin, 2002). According to Dix et al. (2009), biological control with native natural enemies and or coevolved natural enemies of the invasive pest can be very successful in the new habitat. Even though biological control measures can be very successful, it often requires long-term, meticulous strategic planning and regulations, thus rendering such attempts inadequate in early days of invasion (Strong and Pemberton, 2000). While research is on going with coevolved and native natural enemies, Ruesink et al. (1995) suggest we adopt the policy of 'shoot

first, ask question later'. They further argues that the best way to decrease the probability that an invasive species can become widespread is to eliminate the pest before it has time to become abundant and develop adaptive strategies that may give it competitive advantage over indigenous species, thus the use of insecticides that are fast and available are recommended. Nevertheless, Allendorf and Lundquist (2003) argues that understanding the population biology, genetics and the evolution of any invasive pest is essential in developing efficient management practices for them.

It is against this background that this study was designed to understand the population biology of *C. partellus in situ*, its genetics and evolution biology through molecular studies and to evaluate some insecticides as first line control measures.

3. MATERIAL AND METHODS

3.1. Field studies

3.1.1. Maize field preparation and agronomic practices

A maize field (100m x 25m) was ploughed in the Agriculture and Research Implementation Area of Cukurova University (39°01'50.5" N, 35°21'06.7" E), Adana Province, Turkey. The planting and harvesting schedule is shown in Table 3.1. In 2017, preliminary study was conducted with less parameters measured. Pioneer (Hybrid 4/2013) maize seed was sowed; 15 cm and 30 cm intra and inter raw spacing, respectively, with the help of a tractor-mount sower. Agronomic practices such as fertilization and irrigation were done *ad libitum*. Irrigation was done by overhead sprinklers (emergence – V8) and by furrow (> V). The composite fertilizer NPK (20:20:20) was applied in two regimes; NPK was applied 2 weeks after germination and NPK (15:15:15) was applied 4 weeks after germination in each maize growing seasons. No pesticides were applied in the fields through the course of the study. Three fluorescent lambs (120W) – mounted light traps (Figure 3.1) were set at ground level around the field in a triangle-forming pattern (Figure 3.1). The lambs of the light traps were lit from 6.0 pm to 6.0 am daily.

Table 3.1. Planting and harvesting schedule for maize in 2018 – 2019.

Year	Maize crop	Planting date	Harvesting date
2018	First	20 March 2018	07 August 2018
	Second	27 June 2018	27 October 2018
2019	First	23 March 2019	07 August 2019
	Second	01 July 2019	30 October 2019



Figure 3.1. Light trap and light trap positions used in the field. A- Light trap; B – Light trap set in a triangular position around the field

3.1.2. Sampling of *Chilo partellus*

Weekly observations of *C. partellus* on maize crops and light trap catches of moths at weekly interval were recorded. Maize plants were also observed weekly for stem borer infestation. Planting and harvesting dates varied in the different years due to different rainfall patterns. Sampling started on 29 March and ended on 18 October in 2018. Sampling started on 03 May and ended on 16 October in 2019. Sampling was done between 08.00 am – 10.00 am. Once the first stem borer infestation was recorded, 15 maize stalks were cut randomly from each cardinal division of the field: NW, NE, SW, SE. The stalks were taken to the Entomology laboratory, Department of Plant Protection, Cukurova University,

Adana, Turkey and were meticulously examined. The maize stalks were dissected and the number of *C. partellus* larvae and pupae were counted as well as other stem borers. Data was collected on number of egg masses, pupae, and locations of these biological stages of the stem borers. Egg parasitism, plant growth stages and plant damage were also recorded. Sampling of *C. partellus* is summarised in Figure 3.2.



Figure 3.2. Summary of sampling procedure of *Chilo partellus*. Arrows show larvae or pupae from damaged stems and cobs.

3.1.3. Temperature and humidity data

Daily maximum and minimum temperature and percentage relative humidity (RH%) values were provided by the Meteorological station of Department of Agricultural Structure and Irrigation located at Cukurova University, Agriculture and Research Implementation Area. Daily and weekly mean temperature and RH% values were estimated.

3.1.4. Bionomics of *Chilo partellus*

3.1.4.1. Population dynamics of *Chilo partellus*

3.1.4.1.(1). Population fluctuations of *Chilo partellus*

The mean larvae of *C. partellus* per plant was plotted against the sampling dates. The mean values for *C. partellus*, *O. nubilalis* and *S. nonagrioides* per plant were also plotted. The percentage of maize plant and plant part damaged by stem borers were also recorded as a fractional percentage of the total number of plants sampled.

3.1.4.1.(2). Rate of natural parasitism of egg masses of *Chilo partellus*

The maize leaves were thoroughly examined for egg masses in the laboratory. The egg masses were incubated in the Laboratory of Entomology, Department of Plant Protection, Cukurova University. The egg masses were checked daily under a stereomicroscope (x10) for parasitized egg masses. Days before wasp(s) emergence, parasitized egg masses become black and were easily differentiated from unparasitized egg masses (Woelke et al., 2019). The percentage of parasitism (PP) was estimated as the fraction of the number of parasitized eggs masses (P) of the total number of eggs masses (T) collected per sampling date (equation 3.1).

Equation 3.1. Percentage of natural parasitism (%) of *Trichogramma evanescens* on eggs of *Chilo partellus*

$$PP = \frac{P}{T} \times 100$$

Where PP is percentage of parasitism, T is the total number of eggs masses, P is the total numbert of eggs masses collected per sampling date

3.1.4.1.(3). Statistical analyses

Data for the proportion of plant parts damage according to maize phenology was transformed using the arcsine transformation. A two sample t-test (for the vegetative stage) and a one-way ANOVA (in the mature and dryness stages) was done to ascertain if the proportion of plant parts damaged by stem borers were significantly different at a signicant level of 0.05. Tukey HSD post hoc was used to separate significantly different means. A correlation was conducted for the mean light trap catches and the mean larvae values from the dissected maize plants. All measurements were done with emphasis on the maize plant phenology. Analyses were done using SPSS (IBM SPSS ver 23).

3.1.4.2. Dispersion of *Chilo partellus* - Taylor Power Law

Taylor's Power Law (TPL) was used to determined dispersion (spatial distribution) type of the different life stages of *C. partellus* on different plant parts in different growing seasons. TPL is an accepted and widely used tool in Ecological studies to estimate the spatial dispersion of many arthropod pests (Atakan, 2010; Arruda-Neto et al., 2014; Damos, 2018). TPL provides a robust relationship between the variance and the mean of the population. It assumes that the spatial variance (S^2) has a proportional relationship to the fractional power of the mean (m) population density (equation 3.2), where a and b are constants; a is a sampling parameter affected primarily by sample size (n) and b is the Taylor aggregation index which is a characteristic value of a species (Taylor et al., 1978). TPL can be linearized using the Generalized Linear Model (GLM) logarithmic function as shown in equation 3.3. Depending on the value of the species-specific aggregation index (b), the spatial-dispersion can be described as uniform, random or aggregated (Taylor, 1961) (Table 3.2).

Equation 3.2. Taylor Power Law showing relationship between mean and variance

$$S^2 = am^b$$

Equation 3.3. Logarithmic linearized Taylor Power Law

$$\log S^2 = \log a + b\log m$$

Table 3.2. Spatial distribution decision criteria using Taylor Power Law (Taylor, 1961)

Aggregation criteria	Spatial distribution decision
b < 1	Uniform
b = 1	Random
b > 1	Aggregated/Clumsy

3.1.4.3. Determination of optimal sample size

Taylor's coefficient during all stages of maize were used for the development of enumerative sampling plan for larvae+pupae at precision levels of 0.10, 0.20 and 0.30.

The optimal maize plant sample size was determined using Karandinos (1976) equation (3.4) for different *C. partellus* stages and different growing seasons.

Equation 3.4. Optimal sampling size estimation (Karandinos, 1976)

$$N = \frac{Z}{D^2} \times am^{b-2}$$

Where:

N – sample size

Z - 1.96 for $\alpha = 0.05$

a & b - Taylor's Power law coefficients

m – mean of the population

D – is a fixed precision level which was fixed at 0.10, 0.20, 0.30

3.1.4.4. Prediction of *Chilo partellus* phenology using Degree-Days (DD) models

3.1.4.4.(1). Data for Degree-Days model studies

Data collected in 2017 and 2018 was used to develop the models. The data collected in 2019 was to validate the performance of the models.

3.1.4.4.(2). Temperature data and degree-days (DD)

Daily minimum (T_{min}) and maximum (T_{max}) air temperature data for 2017, 2018 and 2019 were obtained from the meteorological station in the experimental site. The DD accumulated after the 1st of May (when first larvae were recorded) was estimated using the summation equation (3.5).

Equation 3.5. Estimation of accumulated degree-days from daily maximum and minimum temperatures

$$\sum_{1}^{n} DD = \int_{1}^{n} \left[\frac{T_{max} + T_{min}}{2} - T_b \right] dT$$

where DD is the daily degree-days, T_{max} is the maximum daily temperature and T_{min} is the minimum daily temperature, and T_b is the minimum temperature threshold (Damos and Savopoulou-Soultani, 2010). The minimum temperature threshold of 10 ^{o}C for *C. partellus* was adopted from Dhillon and Hasan (2018).

The 1st of May was used as a biofix because it is in this month that the first larvae were observed on the maize plant.

3.1.4.4.(3). Statistical analysis for Degree-Day (DD) model

A simple linear regression was conducted to evaluate the effect of temperature and relative humidity on the number of larvae. Chi-square test was done to determine whether the number of larvae observed in 2017 were significantly different from the observations in 2018. Additionally, the cumulative DD in 2017, 2018 and 2019 were evaluated for significant differences. The data from 2017 and 2018 were used to create the DD models while the data from 2019 was used to validate the DD model. For all analysis, α was 5% with regards to hypothesis testing. Analyses were done using SPSS (IBM SPSS ver 23).

3.1.4.4.(4). Models to predict the first and second peak populations of *Chilo partellus*

Two models were assessed to predict the seasonal peaks of *C. partellus* in maize fields using data of 2019. The cumulative DD (independent variable) and the weekly *C. partellus* count (dependent variable) were fitted into these models using 1 May as a biofix. These nonlinear regression models were used because they are s-shaped types of cumulative distribution functions, suitable for numerical work with parameters. First, 3-parameter 'Boltzman' type regression equation (3.6) was used on the data.

Equation 3.6. 3-parameter 'Boltzman' type regression

$$f(x) = \frac{\alpha}{1 + e^{[(x - x_0)/\beta]}}$$

where, *x* is the accumulated degree days, and α, b and c are constants; a and b is the upper and lower asymptote of the curve, while c is the gradient of the curve and represents the time (DD_{50}) of 50% moth emergence (Damos and Savopoulou-Soultain, 2010).

Secondly, 4-parameter 'Logistic' type regression (equation 3.7) was fitted on the data.

Equation 3.7. 4-parameter 'Logistic' type regression

$$g(x) = d + \frac{\alpha}{1+(x/c)^b}$$

where *g* is the cumulative percentage of *C. partellus* catches, *x* is the accumulated degree-days, and α, b, c and d are constant numerical parameters. These parameters regulate shape of the logistic regression function. Additionally, parameter c indicates the time (DD_{50}) of 50% moth emergence (Damos and Savopoulou-Soultain, 2010; Pehlevan and Kovanci, 2017).

The parameters of these models were estimated using the pooled data of 2017 and 2018. Parameter estimates were based on an iterative ordinary least square (OLS), with the Levenberg-Marquard algorithm, in which the cumulative *C. partellus* numbers were the independent variable (IBM SPSS ver. 23).

A Pearson correlation analysis was computed to assess the relationship between the observed and the predicted population generations by the models.

3.1.4.4.(5). Evaluation of models predictive performance using statistical analysis

For each model, the Adjusted coefficient of determination (Adj. R^2), and an Akaike Information Criteria (AIC) were calculated using the data of 2019 (equation 3.8 and 3.9, respectively)

Equation 3.8. Adjusted coefficient of determination (Adj. R2)

$$Adj\ R^2 = 1 - \frac{\left[\frac{RSS}{n} - (\theta+1)\right]}{\frac{SS}{n} - 1}$$

Equation 3.9. Akaike Information Criteria (AIC)

$$AIC = n \ln\left(\frac{RSS}{n}\right) + 2\theta$$

where, RSS is residual sum of square, n is the number of samples, θ is the number of parameters, SS is the total sum of squares. Adj. R^2 and AIC are usually used to statistically compare the performance and accuracy of models (Akaike 1974; Burham and Anderson. 2002; Damos & Savopouou-Soultani. 2010; Akotsen-Mensah et al. 2011, Pehlevan and Kovancı. 2017). Due to the relatively small sample size and a high number of parameters in the models, the AIC was corrected (AICc) for sample size using equation 3.10.

Equation 3.10. Corrected Akaike Information Criteria (AICc)

$$AICc = n \ln\left(\frac{RSS}{n}\right) + 2\theta + \frac{2\theta(\theta+1)}{(n-\theta-1)}$$

where, RSS is residual sum of square, n is the number of samples, and θ is the number of parameters. In order to select the best model, Delta (Δ) scores of AIC and Akaike weights (Wi) was calculated using equation 3.11 and 3.12 respectively. The weight is backed up by the Δ and AICc in determining the best models.

Equation 3.11. Delta (Δ) scores of Akaike Information Criteria

$$\Delta_i = AICc_i - AICc_{smallest}$$

Equation 3.12. Akaike weights (Wi)

$$Wi = \frac{e^{-0.5\Delta_i}}{\sum_{r=1}^{N} e^{(-0.5\Delta_r)}}$$

where, N is number of regression (r) models.

3.2. Laboratory studies

3.2.1. Morphological identification of egg parasitoid of *Chilo partellus*

3.2.1.1. Insect samples

The egg parasitoids of *C. partellus* were collected from field populations of *C. partellus* eggs in 2018 and 2019. Once a week between 08.00 – 10.00 am, 60 maize stalks were selected randomly and cut from ground level and transported to the Laboratory of Entomology in the Department of Plant Protection, Cukurova University. The leaves were meticulously examined for egg masses. The egg masses of *C. partellus* were incubated and the egg parasitoids that emerged were identified. Preliminary studies conducted in 2017 showed it gets easier finding parasitized egg masses of *C. partellus* in August and September, when there is a peak population and the presence of other lepidopteran stem borer pests. Thus, much emphasis in this study was during this period. About 185 egg masses (each containing 35-50 eggs) of maize stem borer *C. partellus* were collected within this period. About 15 females and 10 males of *Trichogramma* sp. were reared from these parasitized eggs. These newly hatched egg parasitoids *Trichogramma* sp. were isolated and supplied with a large number of eggs of laboratory host, moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) attached on paper cards. So

far, a colony of egg parasitoid, *Trichogramma* sp. is maintained at the aforementioned laboratory (25.0 ± 2 °C, 68 ± 2 RH%, 16L:8D h photo-period) on eggs of *E. kuehniella*. Although *E. kuehniella* is a very destructive pests of stored grains (Tarlack *et al*., 2015), its eggs and larvae are widely used as conventional host for laboratory rearing of many entomophagous insects (Samara et al., 2008). Two samples containing over 200 dead adult specimens of *Trichogramma* sp. from laboratory culture were shipped to Dr. Victor Fursov for detailed morphological study and species identification. Dr. Victor Fursov is a taxonomist, stationed at the Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kiev, Ukraine).

3.2.1.2. Species identification

Standard methods of slide mounting were used for the preparation of micro-slides on glasses (Pinto, 1999; Platner et al., 1999). About 200 specimens of *Trichogramma* sp. were macerated in 10% liquid of potassium hydroxide (KOH), then washed in distillated water and mounted in Faure's liquid on glass slides under small cover slips. Thirty males of *Trichogramma* were dissected, then slide-mounted and used for the identification. Microscope Olympus CX-40 with digital photo-camera Olympus CX4040 was used to receive digital photos of morphological characters. Voucher specimens mounted on micro-slides are deposited at the Department of entomophagous insects and biocontrol, Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kiev, Ukraine).

3.2.2. Evaluation of some biological parameters of Trichogramma evanescens on eggs of Ephestia kuehniella at three constant temperatures

3.2.2.1. Rearing of insect species

The *Trichogramma* sp. collected on eggs of *C. partellus* has been identified by Dr. Victor Fursov as *Trichogramma evanescens*. In this part of the study, two insects were used; *Trichogramma evanescens* and the Mediterranean Flour Moth (MFM) *E. kuehniella* Zeller (Lepidoptera: Noctuidae), the latter as the host insect. The initial population of the MFM was obtained from the Biological Control Research Institute in Adana in 2016. *Ephestia kuehniella* is maintained on a diet of corn flour: wheat flour: wheat bran (2:1:1) mixture, in climate rooms at 25°C, 60% R.H and 14:10 (light: dark). The *T. evanescens* used in this study was collected from field collected eggs masses of *C. partellus* in the Agricultural Research and Implementation Area, Cukurova University in 2018. The parasitoid was maintained on UV-sterilized (1.5hr) egg masses of MFM. A non-toxic, solvent-free and transparent glue (GlueFix Kores®) was smeared on a white card (7 cm x 1 cm). The sterilized eggs were spread on the smear and allowed to dry for 1 hour. The cards were then supplied to newly hatched adults of *T. evanesens* for oviposition in 10 cm x 1.5 cm (height x diameter) glass test tubes (Figure 3.3). The setup was kept in a insect rearing cabin (25 ± 2 °C, 65 ± 5 RH% and 14:10 Light:Dark photperiod). The MFM and *T. evanescens* colony are being maintained in the laboratory of Industrial Plant Pest.

3.2.2.2. Evaluation of parasitism capacity

A pair of *T. evanescens* newly emerged male and female (< 12hr) were put in transparent glass vial (10 cm x 1 cm diameter). A cardboard (Trichocards) (2 cm x 1 cm) containing 50 MFM UV-sterilized egg glued on it was added in the glass vials. Fifty eggs of MFM was used because the average number of eggs per egg mass of *C. partellus* from the observations was 50. Also, this study was carried out to optimize the rearing of this parasitoid, which was unidentified at the time of this

study. A drop of diluted honey was put on the inner wall of the glass vial as food for adult parasitoids since most adult parasitoids need food such as nectar, pollen, or honey dew and some feed on body fluids of the host, in contrast others require free water as adults (DeBach and Rosen, 1991; Altieri and Nicholls, 1998). The glass vials were covered with a mesh cloth and tightened by a rubber band (Figure 3.3). The cardboard was replaced daily after parasitization until the dead of the adult female. The setup was kept in an insect rearing cabin (Nuve TK120) with conditions of X ± 1 °C, 65 ± 5% RH, and 14:10 Light:Dark photoperiod, where X was the various constant temperature investigated; 20, 25 and 30 °C. There were 15 replications per treatment. The eggs were monitored every day until hatching. Parasitized eggs turned black 6 – 7 days after parasitization (Flanders, 1937).

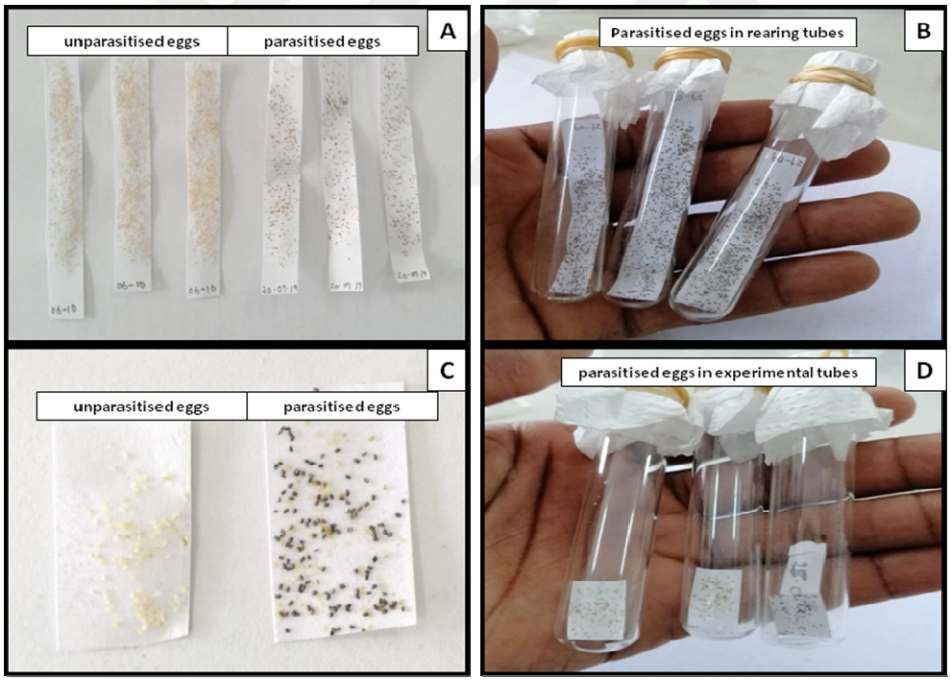


Figure 3.3. Set up for rearing *Trichogramma evanescens* and parasitism on UV-sterilized eggs of *Ephestia kuehniella*. A and B – setup for colony maintenance; C and D – experimental setup with Tricho cards

3.2.2.3. Evaluation of fecundity and fertility capacity of *Trichogramma evanescens* on eggs of *Ephestia kuehniella*

The number of eggs laid by female *T. evanescens* was counted under a light microscope (X10) (OLYMPUS SZ-ILST). This was possible on day 5 – 6 after oviposition, as the colour of parasitized eggs change from cream white to brown and eventually dark; indicating larval development. The number of eggs laid was a measure of fecundity capacity of *T. evanescens* on UV-sterilized eggs of *E. kuehniella*.

Upon hatching, the number of unhatched larvae of parasitoid was counted under the microscope. The proportion of hatched larvae was considered as the fertility capacity of *T. evanescens* on UV-sterilized eggs of *E. kuehniella*.

3.2.2.4. Longevity of Trichogramma evanescens adults on eggs of Ephestia kuehniella

Parasitized egg masses were transfered into a glass vial (10 cm x 1 cm diameter) and covered with a mesh cloth and fastened by a rubber band. The number of days from oviposition to hatching of parasitoid was recorded. This was described as egg-adult period. The setup was kept in an insect rearing cabin (Nuve TK120) with conditions of X ± 1 °C, 65 ± 5% RH, and 14:10 Light:Dark photoperiod, where X was the various constant temperature investigated; 20, 25 and 30 °C. The eggs were monitored every day until hatching. On the day of hatching, two adult, male and female were transfered into clean glass vial. A drop of diluted honey was put on the inner wall of the glass vial as food for adult parasitoids. The glass vial was covered with a mesh cloth and fastened by a rubber band. The setup was kept in the same condition as was in the egg-adult period experiment. There were 15 replications per treatment. The longevity of adult male and female was recorded. In order to assess adult longevity, the adults in the test tubes where view under the microscope; if no movement was observed for about

30 seconds, the test tube was gently shakened and tilted slightly. If no further movement was observed, the adult was considered dead.

3.2.2.5. Data collection and analyses

Data was collected on many parameters; fecundity, parasitism rate (percentage of parasitism), developmental time of egg-adult period, percentage hatching, longevity of males and females, and sex ratio (female/male)

One-way Analysis of Variance (ANOVA) was done to investigate significant different effect of temperature on these parameters. Before ANOVA, data was investigated for normality using Kolmogorov-Smirnov test and homogeneity of variance using Levene's test. The null hypotheses for normality and homogeneity test were not rejected, hence Analysis of Variance. Where means were significantly different, Tukey HSD post hoc test was used to separate means. In addition, a two sample t-test was conducted to evaluate proportion of male and females within each constant temperature. All analyses were done at $\alpha = .05$ using SPSS (ver. 23).

3.2.3. Susceptibility of *Chilo partellus* to some selected insecticides

3.2.3.1. Rearing of *Chilo partellus*

The stem borer colony used in this study was the F1 generations of field-collected larvae of *C. partellus*. In June 2018, larvae were collected from non-insecticide sprayed maize field in the Research and Implementation Area of the Department of Plant Protection, Çukurova University. The larvae were reared on insecticide-free maize stalks (Pioneer Hybrid 1/2013) in the laboratory. The maize stalks were cut (7 cm) and packed in a plastic cup (10 x 10 x 6 cm). The lid of the plastic cup was replaced with a mesh material for ventilation purpose (Figure 3.4). The maize stalks were replaced every 5-6 days until pupation. Upon pupation, the pupae were kept in new plastic jars (12 cm high x 6 cm diameter) lined with a Whatman paper for oviposition and covered with a muslin mesh and tightened by a

rubber band. A cotton ball soaked in water was added in the plastic jar for the adults. The setup was kept in the rearing cabin (27 ± 1 °C, 65 ± 5% RH, 14:10 Light:Dark photoperiod) (Figure 3.4). Egg masses were laid on the Whatman paper lining the walls of the container (Figure 3.4). The egg masses laid were collected daily for bioassays. The eggs (< 24 hr old) and larvae (< 24 hr old) from these F1 generations were used in the study.



Figure 3.4. Setup for maintaining a colony of *Chilo partellus*.

3.2.3.2. Insecticides used in the bioassay

Table 3.3 shows the list of selected insecticides commonly used in maize agro-ecosystem (Kurtuluş and Kornoşor, 2015; Kurtuluş, 2016) or recommended for other lepidopteran stem borers in Turkey. The insecticides ranged from organophosphates to novel insecticides like benzoylphenyl ureas. Pesticides were used at the manufacturer's recommendation for other lepidopteran stem borer pests of maize, diluted in distilled water. Topical and leaf-dip bioassay were used for the hatchability and mortality studies, respectively.

Table 3.3. Tested insecticides; description, formulations and uses

Active ingredient (a.i)/ Trade name	Con./ Form	Field rate (a.i.%)*	Chemical class	Mode of action	Crops	Target pests
Spinosad/ LaserTM	480 gL^{-1}/ SC	20 $mlda^{-1}$	Spinosyn	Ingestion and contact. Nicotinic acetylcholine (nAChR) receptor agonist. Nerve action	Tomato, eggplant, pepper, potato, strawberry, legumes,pome fruits, vegetables, ornamental plants, grass	Lepidoptera, Coleoptera, Diptera
Deltamethrin/ Decis®	25 gL^{-1}/ EC	500 $mlha^{-1}$	Pyrethroid	Ingestion and contact. Paralyses nervous system; knockdown effect.	Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut	Mites, thrips, aphids, Lepidoptera, Coleoptera
Lambda-cyhalothrin/ Karate®	50 gL^{-1}/ CS	300 $mlha^{-1}$	Pyrethroid	Ingestion and contact. Paralyses nervous system; knockdown effect.	Maize, cotton, apple, grapes, potatoes, beet, tomatoes, nuts, cabbage, cereals, olive	Lepidoptera, mites, aphids,
Chlorantraniliprole/ Coragen®	200 gL^{-1}/ SC	150 $mlha^{-1}$	Anthanilic diamide	Ingestion. Ryanodine receptor modulator	Tomato, eggplant, pepper, cucurbit crops, leafy vegetables, maize, peach, quince, plum	Lepidoptera
Indoxacarb/ Tunchii®	150 gL^{-1}/ SC	300 $mlha^{-1}$	Ozadiazine	Ingestion and contact. Voltage-dependent sodium channel blocker	Tomato (field and greenhouse), hazelnut, maize	Lepidoptera
Chlorpyrifos-ethyl/ Dursban®4	480 gL^{-1}/ EC	180 $mlda^{-1}$	Organophosphate	Ingestion and contact. Inhibits cholinesterase. Nerve action	Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut, cotton	Lepidoptera
Emamectin-benzoate/ Pancart®	5%/ SG	300gha^{-1}	Avermectin	Ingestion. Chloride channel activator.	Pepper, tomato (field and greenhouse)	Lepidoptera
Novaluron/ RIMON SUPRA®	100 gL^{-1}/ SC	400gha^{-1}	Benzoylphenyl urea	Ingestion and contact. Insect Growth Regulator – inhibits chitin synthesis.	Tomato (field and greenhouse), pepper (greenhouse), cotton, soya, cucumber	Lepidoptera

Con. – concentration, Form. – Formulations, SC – Suspension concentrate, SG – Soluble granules, EC – Emulsifiable concentrate, CS – Capsule suspension. *These are manufacturer's recommendations

3.2.3.3. Effect of insecticides on hatching potential of 'blackhead' egg masses of *Chilo partellus*

Since the amount of insecticide needed was very small, and to avoid wastage, a 1L stock solution (x10) for each insecticide was prepared using normal tap water. From the stock solution, the recommended doses were prepared to a volume of 0.5 L. Tap water was applied as a control treatment. With the help of micropipette, a drop (5µl) of insecticide was dropped on an egg mass (≈ 30 – 40 eggs) when the egg masses were at the 'black head' stage (≈ 4-5 days old), after which the eggs usually hatch within 24 hours. The egg mass was placed on a maize leaf (2.5 cm x 2 cm), and then placed on a water-soaked cotton in a plastic cup (d = 5 cm, h = 2.5 cm). A hole (d = 1 cm) was perforated on the lid of the cup, and covered with a muslin mesh. There were three replications per treatment. The setup was kept in a rearing cabin (27 ± 1 °C, 70% RH, 14:10 Light:Dark photoperiod) (Figure 3.5). The number of eggs hatched was counted 24 hours after insecticide application.

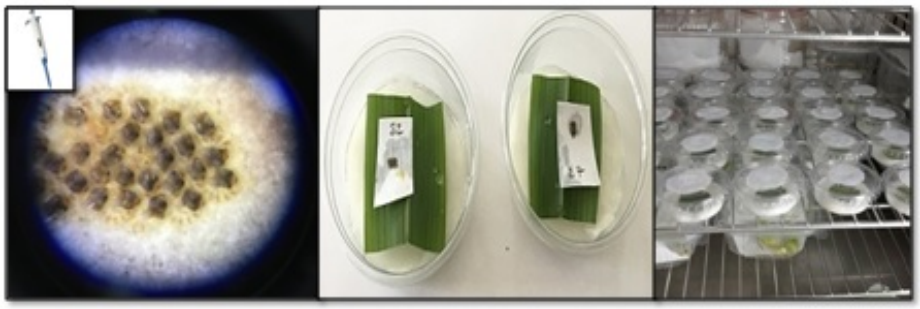


Figure 3.5. Setup for evaluating the effect of insecticides on hatching of 'blackhead' egg masses of *Chilo partellus*.

3.2.3.4. Effect of insecticides on survival of hatched larvae of *Chilo partellus* after 48 hours

The first instar larvae hatched from insecticide-treated eggs were kept separately on fresh insecticide-free maize leaves discs. The leave disk was kept on moist cotton in a plastic cup (diameter = 5 cm, height = 2.5 cm), and then covered with a perforated lid, sealed with a muslin mesh for ventilation. There were three replications per treatment. The setup was kept in the temperature cabin (27± 1 °C, 70% RH, 14:10 Light:Dark photoperiod). Mortality of the emerging larvae was monitored after 48 hours.

3.2.3.5. Effect of insecticides on first instar larvae of *Chilo partellus*

Leaf-dip bioassay was used to evaluate the effect of insecticides on first instar larvae of *C. partellus*. Fresh maize leaf disks (diameter = 2 cm) were immersed in insecticide solution for 5 seconds, and then allowed to dry for 1 hour in laboratory conditions (25 ± 2 °C). Tap water was used as control treatment. The leaf disks were then placed on moist cotton in cups and then covered with a perforated lid, sealed with a muslin mesh for ventilation. Ten first instar larvae of *C. partellus*, collected from F1 generation of laboratory reared field-collected larvae were placed on the leaf disk with the help of a fine camel hair brush. There were three replications per treatments. The setup was kept in the climate cabin (27± 1 °C, 70% RH, 14:10 Light:Dark photoperiod) (Figure 3.6). Mortality of the larvae was monitored every 12 hour for 3 days (72 hrs). A larva was considered dead if it did not move after being touched by a fine camel hair brush. Feeding behaviour of the first instar larvae after 72 hour was evaluated. The number of feeding patchings on the leave was countered and ranked on a four scale from 0 to 3; 0 - no patch, 1 – 1 to 3 patches, 2 – 4 to 6 patches, and 3 – 7 to 10 patches on leaves.



Figure 3.6. Experimental setup for evaluating the effects of insecticides on survival of first instar larvae of *Chilo partellus*.

3.2.3.6. Data analysis

Normality and Homogeneity of Variance tests for the data were done using Kolmogorov-Smirnov (K-S) and Levene's test, respectively. Data of percent hatchability of 'black head' eggs after 24 hours were subjected to one-way Analysis of Variance (ANOVA) and means were separated by post hoc Tukey Honestly Significantly Difference Test (Tukey HSD) procedure at a significance level of .05. Mortality of emerged larvae was monitored after 48 hours of hatching: Chi-square goodness of fit test was used to compare proportions of dead and alive larvae within the insecticides. Mortality of hatched larvae after 48 hours and first instar larval percentage mortality on insecticide-treated leaves after 72 hours was analyzed (one-way ANOVA, Tukey HSD, $P < 0.05$). The mortality was also monitored every 12 hour for survival analysis.

Survival analysis, which is generally a set of methods for analyzing data where the outcome variable is the time until the occurrence of an event of interest (mortality), was conducted. Kaplan-Meier survival curves were used to estimate Median survival time (LT_{50}) of first instar larvae after 72 hours of exposure to the insecticides and their respective 95% confidence interval (CI) was determined. Hazard ratios after 72 hours were estimated using Cox Regression.

At the end of the study (72hr), the number of patches on the insecticide-infested leaves were counted and scored. The scored values were subjected to Kruskal-

Wallis ANOVA. Significantly different mean ranks were separted by Dunn's test ($\alpha = .05$). The percentage of foliar damage reduction (R%) relative to the control was calculated using equation 3.13 (Hannig et al., 2009).

Equation 3.13. Percentage of foliar damage reduction (R%)

$$R\% = \left(1 \quad \frac{T}{C}\right) \times 100$$

where T is the mean score of patches on leaves treated with insecticides and C is the mean score of patches on leaves in the control treatment (water) after the study period.

Based on the parameters evaluated in this study, a hierarchical cluster analysis was conducted in order to group the insecticides based on similarity. All analyses were done using Statistical Package for Social Sciences SPSS (ver. 23).

3.2.4. Molecular studies

3.2.4.1. Sample collection

Chilo partellus samples for molecular studies were collected from the Mediterranean belt of Turkey. In the month of July 2019, four Provinces in the Mediterranean regions of Turkey were visited. In each region, four farmer fields, each from a community were randomly selected, and *C. partellus* were collected. In each field, maize stalks were cut from ground level and larvae (in some cases pupae) of *C. partellus* were collected. The larvae were taken to the Entomology laboratory in Cukurova University. The larvae were feed maize stalks (as described above) until adults emerged. The adults were properly identified as *C. partellus* before any further studies. The samples were stored in a refrigerator (10 °C). Information on latitude and longitude was used to create map showing the aerial view of the sample collection sites (Figure 3.7). The map was developed using

Google® Map online application. Data was also collected on the District, location, plant stage and date of collection (Table 3.4). Four *C. partellus* samples were collected from Cape Town, South Africa by Prof. Van den Berg Johnnie (North West University).



Figure 3.7. Map of the Mediterranean Region of Turkey (Turkey is superimposed) showing collection sites of samples of *Chilo partellus* for molecular studies.

Table 3.4. Locations in the Mediterranean region of Turkey where *Chilo partellus* was collected in 2019

Province	District	Location	Code	Plant stage	Date	Latitude (N)	Longitude (E)
Adana	Sarıçam	Cukurova Uni.	1	Maturity	10-07	37°01'50.5''	35°21'42.1''
Adana	Seyhan	Şakirpaşa	2	Maturity	12-07	37°00'08.3''	35°15'11.7''
Adana	Seyhan	Real	3	Maturity	12-07	37°01'22.6''	35°14'30.3''
Adana	Karabük	Özlüce	4	V6	01-07	37°00'23.4''	35°09'49.8''
Hatay	Kırıkhan	Kırıkhan yolu	1	V4-5	05-07	36°28'53.4''	36°22'14.9''
Hatay	Kırıkhan	Kazkeli Köyü	2	V7-8	05-07	36°25'23.6''	36°22'29.6''
Hatay	Antakya	Demirköprü Köyü	3	V8-9	05-07	36°17'04.4''	36°24'39.0''
Hatay	Antakya	Cilvegözü yolu	4	V4-5	05-07	36°15'17.6''	36°18'51.2''
Mersin	Tarsus	Yenice	1	V8	01-07	36°95'95.5''	35°00'95.2''
Mersin	Tarsus	Yenice	2	V6-7	12-07	37°00'36.6''	35°06'23.9''
Mersin	Tarsus	Yenice	3	V8-9	12-07	37°01'03.3''	35°06'19.5''
Mersin	Tarsus	Yenice	4	Maturity	12-07	36°90'74.4''	34°88'21.4''
Osmaniye	Toprakkale	Toprakkale	1	Maturity	01-07	36°99'90.1''	35°94'20.5''
Osmaniye	Toprakkale	Toprakkale	2	V8	01-07	37°03'14.1''	36°08'45.5''
Osmaniye	Toprakkale	Osmaniye-Ceyhan yolu	3	Maturity	01-07	37°02'53.6''	35°72'07.2''

3.2.4.2. DNA extraction

Genomic DNA was extracted from individual legs of adults of *C. partellus* using the NucleoSpin® DNA Insect Tissue kit (Macherey-Nagel) for genomic DNA from insects. One insect leg was put into a NucleoSpin® Bead Tube Type D and 100 µL Elution buffer (BE). In order to lyse the sample, 40 µL MG buffer was added to the tube, followed by 10 µL Liquid Proteinase K. The tube was closed and agitated for 10 min on a vortex mixer (30 Hertz). The set up was centrifuged for 30 s (11000 x g) in order to clean the lid. In order to adjust the DNA binding conditions, 600 µL MG was added and agitated for 3 s on a vortex mixer (30 Hertz). The set up was centrifuged for 30 s (11000 x g). The supernatant (~ 500 µL) was transfered into the NucleaSpin® DNA Insect Column (2 mL) and centrifuged for 30 s (11000 x g). The tube with the flow was discarded and the column put into a fresh collection tube (2 mL). The silicon membrane was washed

twice. For the first wash, 500 µL BW buffer was added into the column and centrifuged for 3 s (11000 x g). The collection tube with the flow was discarded and replaced. For the second wash, 500 µL B5 buffer was added to the column, centrifuged for 30 s (11000 x g) and the collection tube with the flow was discarded. The silicon membrame was dried by centrifuging it for 30 s (11000 x g). The NucleoSpin® DNA Insect Column was placed into a 1.5 mL nuclease-free tube and 100 µL elution buffer (EB) was added onto the column, incubated at room temperature for 1 min and centrifuged for 30 s (11000 x g). The extracted DNA was stored at – 20 °C until required for amplication.

3.2.4.3. DNA amplification - Polymerase Chain Reaction

The Polymerase chain reaction (PCR) amplications were done in 25 µL volume, containing 5 µL of DNA and 20 µL master mix (Table 3.5). PCR was done using the following conditions: denaturation at 95 °C for 11 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s, and 72 °C for 7 min, and a final hold at 4 °C giving a total time of 2 hr 10 min. The primers used in this study to amplify the cytochrome c oxidase subunit 1 (COI) mitochondrial DNA (mtDNA) gene are given in Table 3.6.

Table 3.5. Contents of the master mix for DNA amplification

Contents	**Quantity (µL)**
Taq buffer	2.5
$MgCl_2$	1.5
dNTP	0.5
LCO1490	1.0
HCO2198	1.0
Taq	0.2
H_2O	13.3

Table 3.6. Primers used in the amplication of mitochondrial COI gene

	Primer (5'_3')	Reference
Forward	LCO1490 (GGTCAACAAATCATAAAGATATTGG)	Folmer et al. 1994
Reverse	HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA)	Folmer et al. 1994

3.2.4.4. Gel electrophoresis

In order to confirm the successful amplication of mtDNA, 5 µL aliquot of the amplification product was loaded in the wells made from agarose gel. The gel wells were prepared by heating a mixture of 0.3 g agarose gel and 20 mL TAE (Tris-acetate-edta) buffer for 2 – 3 min with approximate 5 sequential hand agitation (3 – 5 s). The gel was poured into the casting tray and allowed to cool and solidify for 30 min. The solidified agarose gel slap with a row of wells was transfered into the electrophoresis chamber filled with TAE buffer. The first gel well was loaded with 4 µL of DNA ladder (100bp, H3 RTU) and the other gel wells were loaded with 4 µL of mtDNA of insect samples. The power was turned on the DNA migrated throw the gel. The power was turned off after ~ 45 min, when the blue dye in the DNA had migrated more than 4/5th of the gel. The gel was transfered into an ethidium bromide solution and allowed there for about 2 min. The ethidium bromide stained gel was was exposed to UV light and a photo taken.

3.2.4.5. DNA sequencing

The mtDNA extracted from each sample was sent to Macrogen Inc. South Korea. Macrogen Inc. is a biotechnoloy company located in Seoul, South Korea, and it specialises in DNA sequencing and Bioinformatic analysis.

3.2.4.6. Molecular analysis

The mtDNA chromatograms were edited and assembled using BioEdit (Hall, 1999). In order to remove low quality sequence, the DNA sequences were

trimmed on 5' and 3'. The purpoted identities of each sequence were determined by comparisn with the DNA barcode sequence repository of NCBI database (https://www.ncbi.nlm.nih.gov/). The sequences were aligned using ClustalX (Thompson et al. 1997). Furthermore, some reference sequences from the GenBank of NCBI database (Table 3.7) were downloaded and used in the phylogenetic analysis. A maximum likelihood (ML) analysis based on Kimura-2-parameter model (K2P) for pairwise distance estimation (Kimura, 1980) was used to construct the phylogenetic tree. Gaps in the DNA sequences of the samples were completely removed and clade support was estinated using 1000 bootstrap replicates of the sequences generated in this study and the GenBank (reference sequences) using MEGAx. *Ostrinia nubilalis* was also obtained from the GenBank and used as an outgroup.

Table 3.7. Identified species and their accession numbers from GenBank

Accession numbers	**Species**	**Host plant**	**Country**
KX351380.1	*Chilo partellus*	Maize	India
MK566473.1	*Chilo partellus*	Maize	Kenya
HQ991339.1	*Chilo partellus*	Maize	Pakistan
KP233794.1	*Chilo partellus*	Maize	India
HQ991286.1	*Chilo partellus*	Maize	Pakistan
HQ991263.1	*Chilo partellus*	Maize	Pakistan
HQ991218.1	*Chilo partellus*	Maize	Pakistan
HQ990908.1	*Chilo partellus*	Maize	Pakistan
HQ990905.1	*Chilo partellus*	Maize	Pakistan
HM875015.1	*Ostrinia nubilalis*	Maize	Finland

4. RESULTS AND DISCUSSION

4.1. Population dynamics of *Chilo partellus*

4.1.1. Population fluctuations of *Chilo partellus* and other major stem borers

The population fluctuation of *Chilo partellus* (larvae) in insecticide-free maize field according to maize phenology: vegetative, tassel+cob (mature) and dryness stages for 2018 and 2019 are shown in Figure 4.1. In 2018, sampling began on 29 March for the first crop of maize and ended on 02 August (Figure 4.1a). A major peak population of 6.1 of *C. partellus* larvae was recorded on 10 May. A small (minor) peak of 2.73 *C. partellus* was observed on 28 June. The first observation of *O. nubilalis* and *S. nonagrioides* was on 28 June and 19 July, respectively. The mean population of *O. nubilalis* and *S. nonagrioides* remained low (below 2 larvae per plant) compared to *C. partellus* through the first crop of maize in 2018. The first observation of *C. partellus* on the second crop of maize in 2018 was on 05 July (Figure 4.1b). A peak of 5.75 *C. partellus* larvae per plant was observed on 5 July. After the peak, the population steadily declined between 1-2.8 larvae per plant until the end of the study. Also, in the second crop of maize of 2018, the population of *O. nubilalis* and *S. nonagrioides* steadily increased from 5 July to 16 August (Figure 4.1b). A peak population of 3.91 larvae of *O. nubilalis* was observed on 16 August. On the same day, a peak population of 3.13 larvae of *S. nonagrioides* was recorded. The population of all three maize stem borers steadily declined after 16 August. In 2019, sampling on the first crop of maize began on 03 April (Figure 4.1c). The first *C. partellus* was observed on 15 May. A major peak population of 0.45 larvae per plant was observed on 28 May. The population declined after that until 31 July, when a small peak of 0.19 larvae per plant was observed. Sampling ended on 07 August with approximately zero observations of *C. partellus*. The first observation of *O. nubilalis* and *S. nonagrioides* on the first crop of maize was on 26 June and 10 July, respectively

(Figure 4.1c). A peak population of 0.37 larvae of *O. nubilalis* was observed on 07 August. The population of *S. nonagrioides* remained low (less than 0.1 larvae per plant) throughout the first crop. In the second crop of maize in 2019 (Figure 4.1d), sampling began and ended on 24 July and 02 October, respectively. A major peak population of 2.27 larvae of *C. partellus* per plant was recorded on 31 July. The population steadily steadily declined after that. The population of *O. nubilalis* rose and peaked on 11 September with a mean of 4.7 larvae per plant (Figure 4.1d). The population of *S. nonagrioides* remained relatively low (near zero larvae per plant) for the entire second crop of maize in 2019.

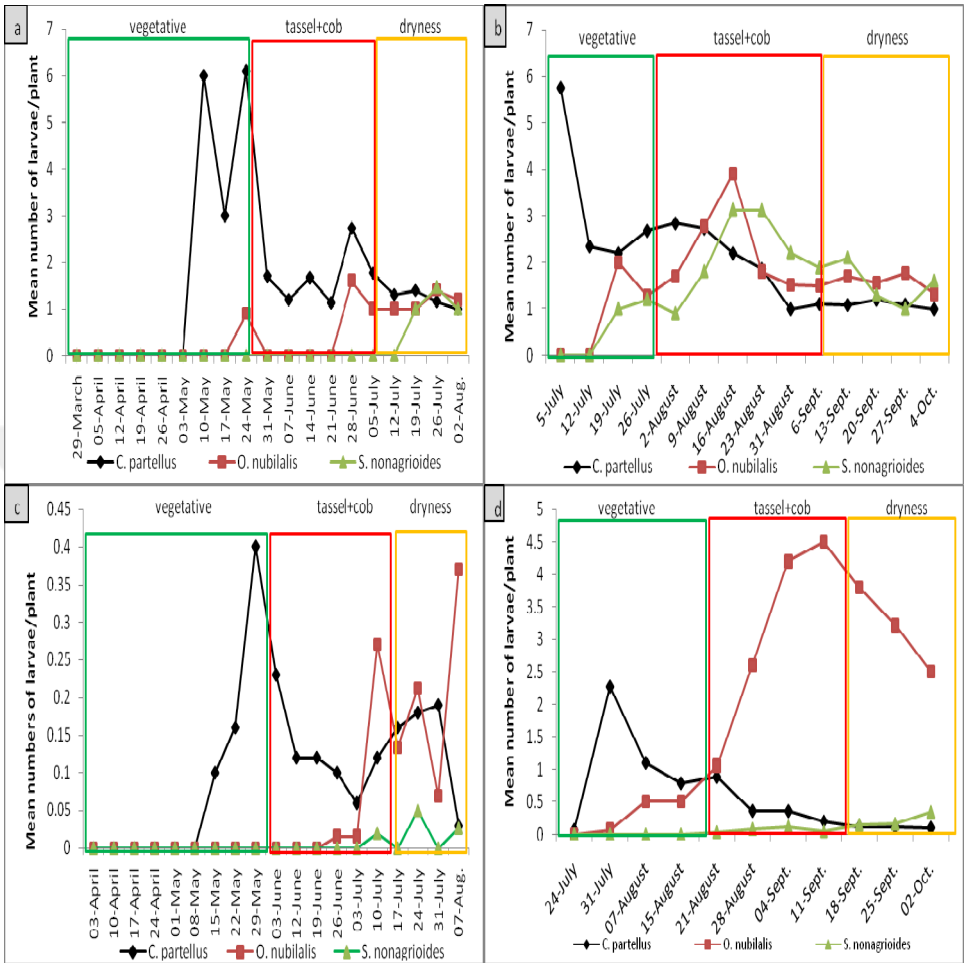


Figure 4.1. Mean numbers of larvae of major maize stem borers on first and second crop of maize according to maize plant phenology in 2018 and 2019. a and b – population fluctuations on first and second crop of maize 2018, respectively. c and d – population fluctuation on first and second crop of maize 2019, respectively. *C. partellus* – *Chilo partellus*; *O. nubilalis* – *Ostrinia nubilalis*; *S. nonagrioides* – *Sesamia nonagrioides*

4.1.2. Population fluctuations of *Chilo partellus* by light trap catches

The mean number of light trap catches of *C. partellus* (adults) on the first and second crop of maize in 2018 and 2019 according to maize phenology is shown in Figure 4.2. In 2018, the first light trap catch on the first crop of maize was on 26 April (Figure 4.2a). The mean number of *C. partellus* captured and peaked (6.3 adults/trap) on 07 June. The light trap catches closely matched the larvae counts on maize stalks. For the second crop of maize in 2018, the first flight was observed on 05 July (Figure 4.2b). Unlike the mean number of larvae on maize stalks with one peak, two distinct peaks, one in the vegetative stage and another in the tassel+cob-dryness interface stage, were observed from light trap catches. In 2019, the first flight on the first crop of maize was on 03 May (Figure 4.2c). Two peaks were observed; one on the vegetative stage and the other on the dry stage of maize. For the second crop of maize in 2019, the first flight was on 24 July with 2.2 adults/trap (Figure 4.2d). A second small (minor) peak occurred on 18 September 2019.

The mean number of light trap catches was regressed with the mean number of larvae on maize stalks for the first and second crop of maize in 2018 and 2019 (Table 4.1). The regression model for the first crop of maize in 2018 (R^2 = 0.234, F = 5.189, P = 0.036) and 2019 (R^2 = 0.547, F = 20.552, P = 0.0001) were significant. On the other hand, the regression model for the second crop in 2018 (R^2 = 0.045, F = 0.560, P = 0.469) and 2019 (R^2 = 0.027, F = 0.249, P = 0.630) were not significant.

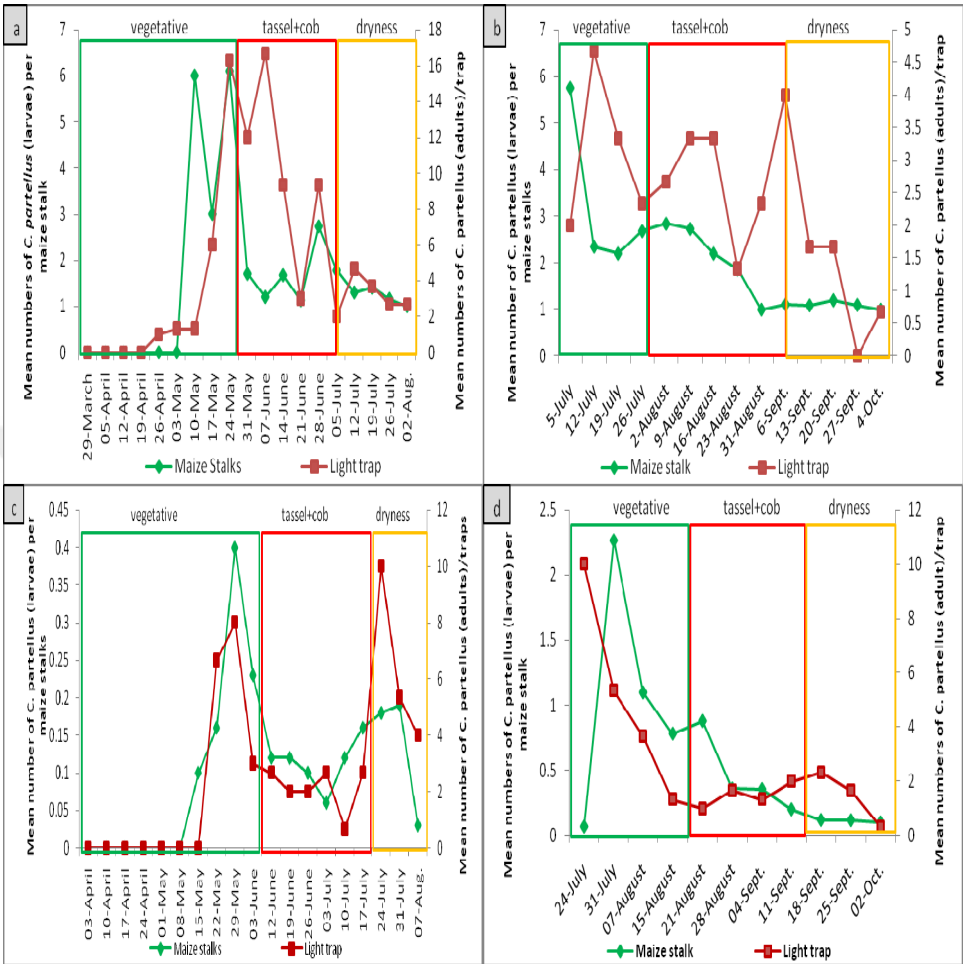


Figure 4.2. Mean numbers of *Chilo partellus* adults (males + females) trapped by the light trap and numbers of larvae obtained from maize stalks from first and second crop of maize according to maize plant phenology in 2018 and 2019. a and b – mean numbers of *C. partellus* on first and second crop of maize in 2018, respectively. c and d – mean numbers of *C. partellus* on first and second crop of maize in 2019, respectively

Table 4.1. Regression analysis between mean numbers of *Chilo partellus* from light trap (adults: males + females) and maize stalks (larvae)

Year	Crop	Regression equation	R^2	F	*P*
2018	First	Y = 1.432x + 2.569	0.234	5.189	0.036
	Second	Y = 0.214x + 1.936	0.045	0.560	0.469
2019	First	Y = 21.256x + 0.41	0.547	20.552	0.0001
	Second	Y = 0.681x + 2.395	0.027	0.249	0.630

4.1.3. Population fluctuations of major stem borers by light traps

The population pattern of major stem borers according to light trap catches on the first and second crop of maize in 2018 and 2019 is shown in Figure 4.3. In the first crop of 2018 (Figure 4.3a) and 2019 (Figure 4.3b), *C. partellus* flight was observed earlier than *O. nubilalis* and *S. nonagrioides*. Flight catches of *O. nubilalis* and *S. nonagrioides* did not exceed 3 adults per traps, whereas that of *C. partellus* peaked on 29 May (16 adults/trap) and 24 July (10 adults/trap) in the first crop of 2018 and 2019, respectively. In the second of maize in 2018, the flight catches of *O. nubilalis* and *S. nonagrioides* were generally higher than that of *C. partellus* (Figure 4.3c). A peak population of 4, 3, and 3 adults/trap was observed on 6 September for *C. partellus*, *O. nubilalis* and *S. nonagrioides*, respectively. In the second crop of maize in 2019, the mean flight catches of *O. nubilalis* and *S. nonagrioides* were generally higher than that of *C. partellus* (Figure 4.3d).

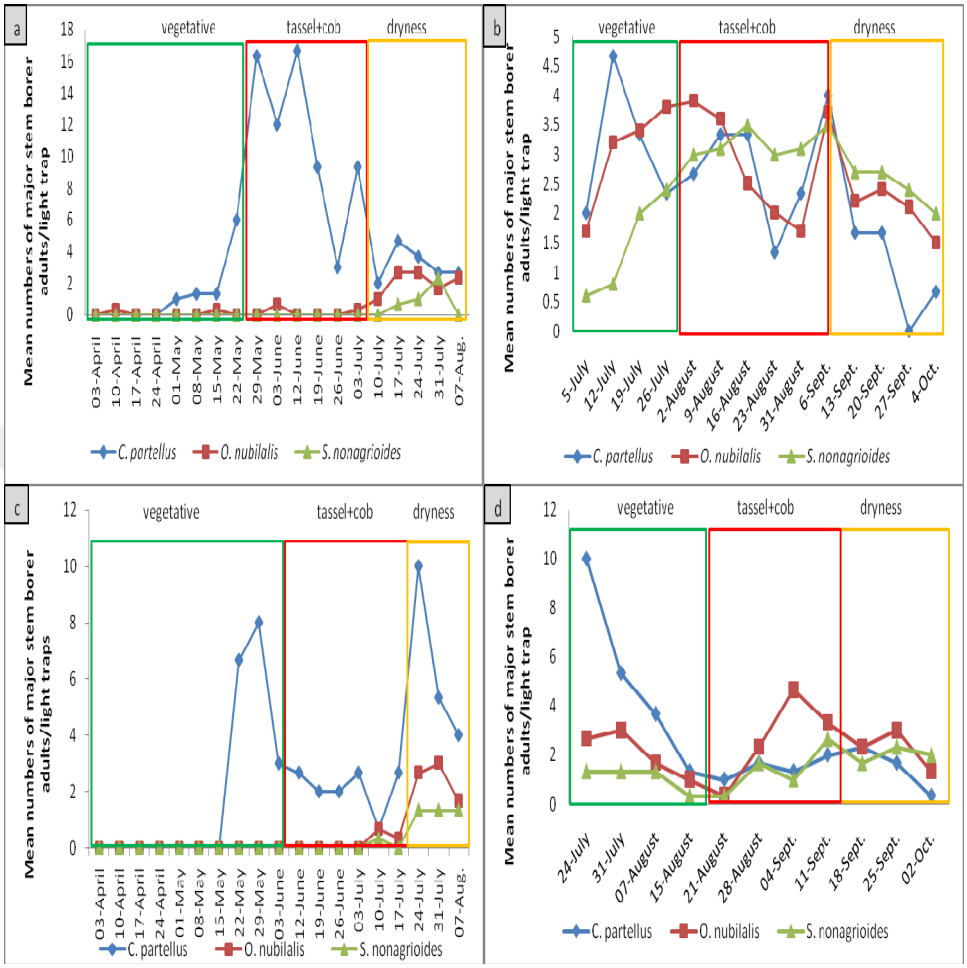


Figure 4.3. Mean numbers of major stem borer adults (males + females) captured by the light traps from first and second crop of maize according to maize plant phenology in 2018 and 2019. a and b – mean numbers of major stem borers on first and second crop of maize in 2018, respectively. c and d – mean numbers of major stem borers on first and second crop of maize in 2019, respectively. *C. partellus* – *Chilo partellus*; *O. nubilalis* – *Ostrinia nubilalis*; *S. nonagrioides* – *Sesamia nonagrioides*

The findings of this study shows that *C. partellus* has two major peak populations per year in the maize agro-ecosystem: first and second peak in the vegetative stages of the first and second crop of maize, respectively. A small

(minor) peak was observed on the dry maize stalks of the first crop of maize. It should be noted that this minor peak was observed at a time when the second crop of maize was at the vegetative stage, and standing nearby. This minor peak in the dry stages of the first crop of maize in the presence of the second crop of maize (at the vegetative stage) could be as a result of overlapping generations. According to Bate et al. (1999), one important characteristics of *C. partellus* population dynamic is the fast population buildup and the occurrence of overlapping generations. It is unlikely that this minor peak observed on the dry stage of the first crop of maize would be observed on farmer fields in Turkey, because Turkish farmers often harvest the first crop of maize earlier than in this study, and prior to when the second crop of maize is planted (Bolatova and Engindeniz, 2018).

It was also observed that *C. partellus* emerged earlier than the two native major stem borers of maize. Early emergence of *C. partellus* is common since it can survive in a wider temperature range and terminates diapause earlier than most maize stem borers (Kfir, 1997a; Dejen et al., 2014). Krüger et al. (2008) also revealed that *C. partellus* infestation levels were high on young maize plants especially in pre-tasseling stages in a study investigating the incidence of major stem borers on maize in South Africa. In the same study, they also observed two major peak populations of *C. partellus*. The finding of Krüger et al. (2008) is in accordance with the finding of the current study.

In Turkey, major stem borers are a real threat in the second crop of maize, causing very severe damages (Sertkaya et al., 2004). This explains why the occurrence of native stem borers: *O. nubilalis* and *S. nonagrioides* populations peaked in the mature stages of maize unlike that of *C. partellus* which peaked in the vegetation stages. Native stem borers of maize seem to have coevolved to infest their host in the mature stages. *Busseola fusca* and *Sesamia calamistis*, which are native stem borers of maize in many subsaharan countries (Ingram, 1983), occur in the tassel and cob (mature) stages of maize (Krüger et al., 2008).

The flight activity of *C. partellus* was observed two weeks (2018) and one week (2019) prior to the first observation of larvae of *C. partellus* on maize plants. The flight activity of *C. partellus* and the mean numbers of *C. partellus* larvae per maize plant fluctuated in a very similar pattern in both the first and second crop of maize and in both 2018 and 2019. Even though both the light trap catches and mean larval numbers per maize plants of *C. partellus* fluctuated in a similar manner, significant correlations were observed only in the first crops of maize of both years, and not in the second crops of maize. The lack of correlation in the second crop of maize could be due to the presence of other major stem borers of maize (*O. nubilalis* and *S. nonagrioides*) in the field, predominantly in the second crop of maize. Other major stem borers of maize compete with *C. partellus* for resources (Kfir et al., 2002), thereby influencing its population, which is reflected in the minimal or lack of synchrony in the populations of the light trap catches and the mean larval count per maize plant of *C. partellus* in the second crop.

In general, the total number of *C. partellus* collected is higher than those of *O. nubilalis* and *S. nonagrioides*. The mean number of *C. partellus* reached 6.1 and 5.7 larvae per plant in the first and second crop of maize in 2018, respectively although the numbers were relatively small in 2019. Krüger et al. (2008) also reported higher *C. partellus* (larvae) number on maize stalks in the pre-tasseling (4.2 larvae/plant) and post-tasseling (2.4 larve/plant) compared to *B. fusca* and *S. calamistis* in semi-arid regions. Similar mean number of *C. partellus* larvae per plant of 3 on maize was also recored by Kfir (1992) in arid regions. Mgoo et al. (2006) investigated the effect of different nitrogen concentrations on the damage and yield of maize by maize stem borers in Tanzania and concluded that of all three stem borers (*Chilo partellus*, *Sesamia calamistis*, and *Chilo orichalcociliellus*) collected in the study, *C. partellus* accounted for 95-98% of the total number. The number of *S. nonagrioides* in the second crop of 2019 in the current study remained near zero. The findings of the current study is in accordance with that of Kurtuluş (2016) who recorded very low mean numbers of *S.*

nonagrioides (0 – 0.4 larvae/plant) on the second crop of maize in Adana, Turkey. *Chilo partellus* is described as an invasive species, displacing native stem borers in many parts of the world (Kfir 1997a, b; Mutamiswa et al., 2017). Krüger et al. (2008) described *C. partellus* as 'dominant species' over *B. fusca* and *S. calamistis* when they found out that a very high percentage of all three stem borers collected was *C. partellus*. Mutamiswa et al. (2017) further states that the numerical advantage of *C. partellus* over native stem borers gives it increased ecological influence, eventually resulting in a dominant status. However, the duration of the current study is not sufficient to conclude that low numbers of *O. nubilalis* and *S. nonagrioides* especially in the first crop of maize are indications of their displacement by *C. partellus*.

Light traps play a very essential role in ecological studies (Southwood, 1978; Sheikh et al., 2016). In the first crop of maize of both years, flight activity started earlier than insect activity on maize stalks. The first flight of *C. partellus* was in May, then declined and peaked again in July and September. A research by Van Hamburg (1979) demonstrated that the flight pattern of *C. partellus* in semi-arid areas peaked during the spring season and late summer on maize and sorghum. The finding of the current study in the Mediterranean region is on a par with that of Van Hamburg (1979). Data from light traps provide very valuable information about the abundance, distribution and the seasonal flight patterns of insects (Walkden and Whelan, 1942). Furthermore, it can also be seen that the *C. partellus* count on maize stalks lagged behind the *C. partellus* light trap catches very closely, in other words, an almost perfect symmetry could be observed between the two observations in the first crop of maize for both years. This pattern was observed on the second crop of maize for both years until the tassel+cob-dryness stage interface, where the light trap catches and the count on the maize stalks were asymmetrical. The observed asymmetry could be due to *C. partellus* adults flying from neighbouring maize and sorghum fields in the research area. This asymetrical relationship probably explains the insignificant regression model observed in the

second crop of maize between the light trap catches and the maize stalk counts in 2018 and 2019. Hendricks et al. (1975) and Conrad et al. (2006) summarised widespread use of light traps in studies of lepidopteran biodiversity monitoring, seasonal flight periods and pest detection. The information in the current study is useful for informed-decision making on management practices of *C. partellus*. It should be noted that migration of these pests from neighbouring maize and sorghum fields in the research area may have influenced the results.

4.2. Maize infestation and plant part damaged by major stem borers of maize

4.2.1. Infestation of maize by major stem borers

The percentage of insectide-free maize plant infested by major stem borers according to maize phenology for the first and second crop of maize in 2018 and 2019 are shown in Figure 4.4. In 2018, the first signs of stem borer infestation on the first crop of maize was observed on 10 May (Figure 4.4a). The percentage increased from 26.67% (10 May) to 40.0% (24 May). The percentage infestation declined steadily and reached 50.0% on 28 June. The highest infestation was in the mature stage of maize. In the second crop of maize in 2018, the percentage increased from 13.3% (5 July) to 96.61% (19 July) (Figure 4.4b). The percentage infestation remained high (above 60.0%) and attained 100% on 31 August. In 2019, the first observation of stem borer infestation on the first crop of maize was observed on 15 May at 3.3% (Figure 4.5c). the percentage infestation remained approximately contant until 17 July, when it reached 57.62%. In the second crop of maize of 2019, the percentage rose from 10.16% (24 July) to 76.27% (31 July) (Figure 4.4d). After 07 August (66.67%), the percentage infestation remained high above 90.0% through the entire study.

The mean numbers of the three major stem borers on maize plant was regressed with the percentage of maize plants damaged by the three major stem borers for the first and second crop of maize in 2018 and 2019 (Table 4.2). In general, positive regression models were observed. The regression model for the

first crop of maize in 2018 (R^2 = 0.615, F = 27.129, *P* = 0.0001) and 2019 (R^2 = 0.703, F = 40.164, *P* = 0.0001), and second crop of maize in 2019 (R^2 = 0.579, F = 12.36, *P* = 0.007) were significant. On the other hand, the regression model for the second crop in 2018 (R^2 = 0.026, F = 0.315, *P* = 0.585) was not significant.

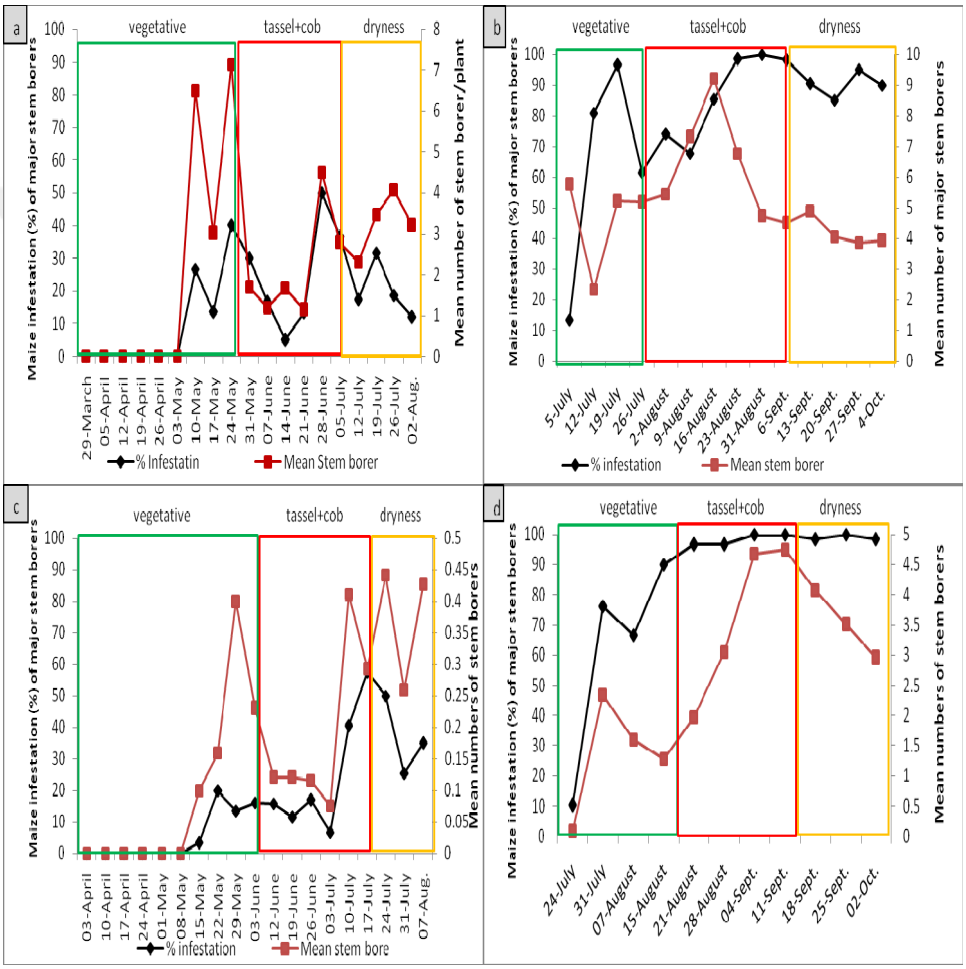


Figure 4.4. Percentage infestation of first and second crop of maize by three major stem borers (*Chilo partellus*, *Ostrinia nubilalis* and *Sesamia nonagrioides*) according to maize phenology in 2018 and 2019. a and b – percentage infestation of maize and mean numbers of major stem borers on first and second crop of maize 2018, respectively. c and d – percentage infestation and mean numbers of major stem borers on first and second crop of maize 2019, respectively

Table 4.2. Regression analysis between mean numbers of three major stem borers (*Chilo partellus*, *Ostrinia nubilalis* and *Sesamia nonagrioides*) larvae from maize stalks and percentage of infestation of maize plant by three major stem borer according to maize phenology and years

Year	Crop	Regression equation	R^2	F	*P*
2018	First	Y = 3.854 + 5.581x	0.615	27.129	0.0001
	Second	Y = 92.556 – 2.16x	0.026	0.315	0.585
2019	First	Y = 1.093 + 92.56x	0.703	40.164	0.0001
	Second	Y = 46.26 + 14.02x	0.579	12.360	0.007

4.2.2. Maize plant parts damage due to infestations by major stem borers of maize

The percentage of plant parts damaged by major stem borers in the first and second crop of maize in 2018 and 2019 according to maize phenology is shown in Figure 4.5. In the first crop of maize in 2018, a t-test revealed that stem borer significantly (t = 8.01, df = 6, *P* = 0.0001) damaged leaves (60.61%) than stems (39.39%) in the vegetative stage (Figure 4.5a). In the tassel+cob stage, the leaves (42.93%) and stems (39.63%) were significantly (F = 14.8, df = 3, 20, *P* = 0.021) more damaged than tassel (4.44%) and cobs (12.96%) (Figure 4.5a). In the dryness stage, stems (29.55%) were significantly (F = 10.12, df = 3, 16, *P* = 0.0113) more damaged compared to the other plant parts (Figure 4.5a). In the second crop of maize in 2018, the damage on leaves (62.35%) was significantly (t = 9.12, df = 6, *P* = 0.038) higher than on stems (37.64%) in the vegetative stage (Figure 4.5b). In the tassel+cob stage, leaves (37.70%) and stem (37.81%) were significantly (F = 7.04, df = 3, 20, *P* = 0.021) damaged more than cobs (21.05%) and tassel (3.44%) (Figure 4.5b). In the dryness stage, the damage on leaves (33.17%) and stem (39.77%) was significantly (F = 21.41, df = 3, 12, *P* = 0.041) higher than those of cob (22.11%) and tassel (4.95%) (Figure 4.5b). In the first crop of maize in 2019, the percentage of damaged leaves (68.00%) was significantly (t =32.01, df = 8, *P* = 0.0001) higher than that of the stem (32.00%) in

the vegetative stage of maize (Figure 4.5c). In the tassel+cob stage, the damage on stem (51.77%) was significantly (F = 16.24, df = 3, 20, *P* = 0.031) higher than those of leaves (26.95%), cob (16.31%) and tassel (8.00%) (Figure 4.5c). In the dryness stage, the percentage of damaged stem (57.41%) was significantly (F = 10.23, df = 3, 8, *P* = 0.04) higher than those of cob (24.07%), leaves (9.26%) and tassel (9.36%) (Figure 4.5c). In the second crop of maize in 2019, the percentage of damaged on leaves (76.21%) was significantly (t = 54.10, df = 6, *P* =0.001) higher than that of stem (23.24%) in the vegetative stage (Figure 4.5d). In the tassel+cob stage (Figure 4.5d), the percentage of damaged on stem and leaves were significantly different (F = 2.04, df = 3, 12, *P* = 0.037) for different plant parts: stem (38.92%), leaves (34.18%), cob (23.18%) and tassel (8.0%). In the dryness stage in the second crop of 2019, the percentage of damage on stems (57.41%) was significantly (F = 14.02, df = 3, 8, *P* = 0.019) higher than of leaves (16.88%), cob (26.69%) and tassel (3.18%) (Figure 4.5d).

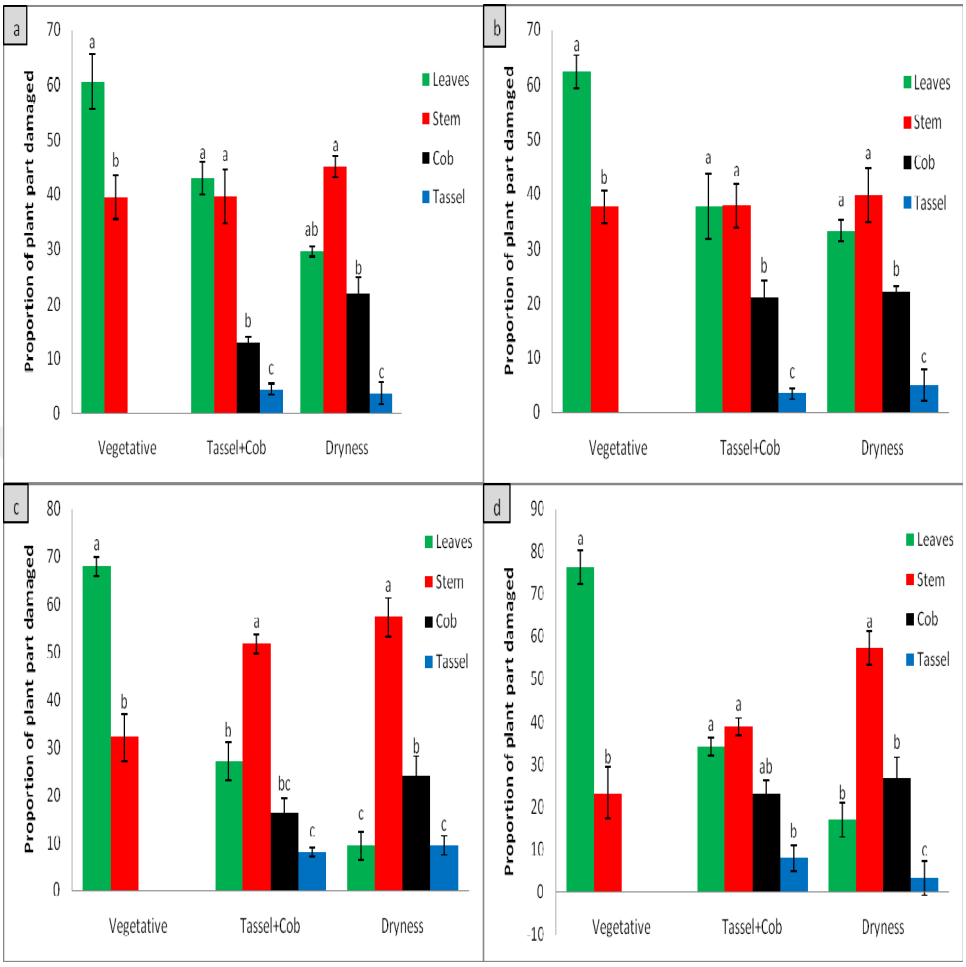


Figure 4.5. Percentage of maize plant parts in the first and second crop of maize damaged by major stem borers at different maize phenology in 2018 and 2019. a and b – percentage of damaged parts on first and second crop of maize in 2018, respectively. c and d – percentage of damaged parts on first and second crop of maize in 2019, respectively

4.2.3. Percentage of dead heart maize plants

A maize plant is described as 'dead heart' when it is severely damaged by the maize stem borers. The stems, leaves and apical meristem are severely damaged resulting into a stunted or dead plant (Figure 4.6). The percentage of dead heart of insecticide-free maize plants in the first and second crop of maize in 2018 and 2019 according to maize phenology is presented in Figure 4.7. In 2018, the

first dead heart plants were recorded on 17 May at 3.33% on the first crop of maize (Figure 4.7a). Two major peak of dead heart occurred on 31 May (8.33%) and 21 June (10.0%) in the tassel+cob stage of maize. In the second crop of maize of 2018, the first dead heart was observed on 19 July at 11.66% in the vegetative stage, and a peaks were sustained on 09 – 16 August at 16.66% (Figure 4.7b). In the first crop of 2019, the dead heart was observed on two dates; 29 May and 03 June at 1.7% and 2.0%, respectively (Figure 4.7c). In the second crop of maize, the first dead heart was observed on 15 August at 8.33% in the vegetative stage and steadily declined thereafter until 11 September (6.67%) in the tassel+cob stage (Figure 4.7d).

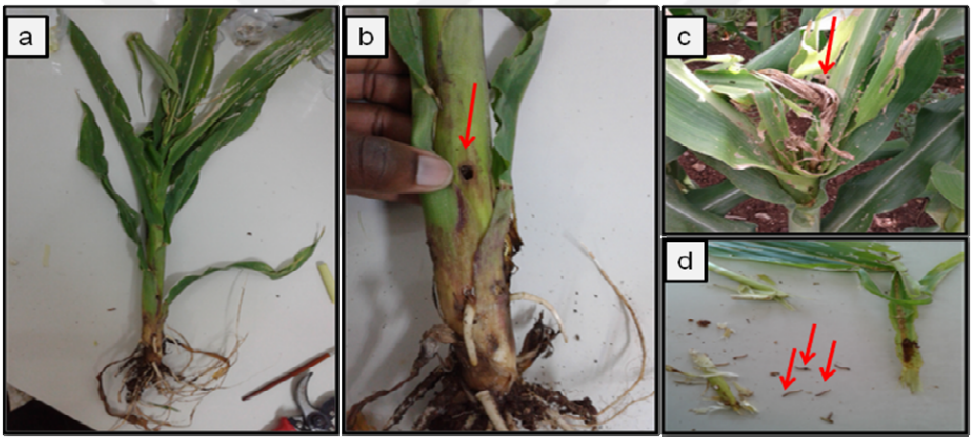


Figure 4.6. Maize plant severely damaged by stem borers resulting in symptoms of dead heart. a – whole plant with damged leaves and stem; b – entry whole on stem created by stem borers; c – damaged apical meristem; d – larvae from damaged whorl.

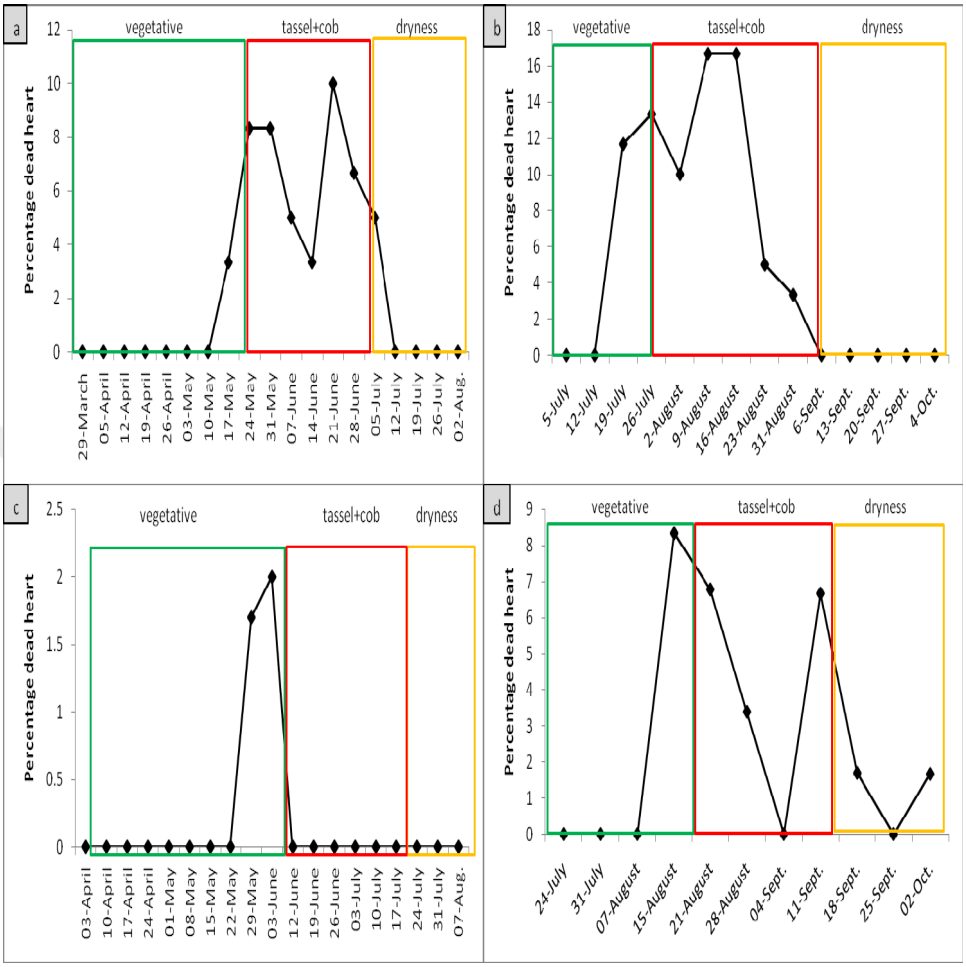


Figure 4.7. Percentage of dead heart of maize plants from first and second crop of maize at different maize phenology in 2018 and 2019. a and b – percentage of dead heart on first and second crop of maize in 2018, respectively. c and d – percentage of dead heart on first and second crop of maize in 2019, respectively

Maize infestation and the percentage of plant pests damaged is considered a result of all the major stem borers of maize because the damage designs and patterns are very similar for all major stem borers (Krüger et al., 2008). Mixed populations of the three major stem borers in this study was not observed prior to mid June. Thus, it is possible to infer that any infestation and damages observed

prior to this date is a result of *C. partellus* infestation, which was the only stem borer recorded at the time. The percentage of maize plant infested prior to late June – early July ranged between 3.3% to 50.0% for both years. According to Bate and Van Rensburg (1992), the economic threshold levels (ETL) for *C. partellus* control is 40.0% of plants exhibiting whorl damage. However, in the current study, the percentage infestation reported is a sum total of whorl, leaf and stem damage. Thus, at this time (prior to late June and early July), *C. partellus* effect on maize may be considered low based on ETL of 40.0%. On the contrary, a related study Seshu-Reddy and Sum (1991) on many maize varieties, concluded that the economic injury level (EIL) for *C. partellus* ranged between 0.66 – 1.41 larvae per plant, which is inline with the findings of the 2018, and close to those of 2019 in the current study. It should be noted that even though the mean number of *C. partellus* in 2018 was very high compared to those in 2019, the percentage infestations are very similar in pattern and size. Some degree of *C. partellus* control is needed both in the first and second crop of maize in the Mediterranean regions.

The percentage of maize plants parts damaged by major stem borers showed more preference for some plant parts based on maize phenology. In all growing seasons in the current study, the leaves were preferred more than the stems in the vegetative stages. However, through the tassel+cob and dryness stages, the damage shifted from leaves to stem. This shift from leaves to stem in the mature and later stages of maize by stem borers could be an evolutionary strategy to ensure that the maize plant survive and sustain the pest population. This notion can be expatiated by the intensed maize stalk attack and damage by major stem borers occurring in the mature stages of maize (Ingram, 1983; Krüger et al., 2008).

Sometimes, the larvae of maize stem borers feed on the basal meristem of young maize plants, causing very stunted growth, no yield and dead of maize plant (Kfir, 1997a; Sylvain et al., 2015). Like the percentage infestation of maize, the proportion of dead heart in the current study cannot be fully attributed to *C.*

partellus. Nevertheless, it could be inferred that the proportion of dead heart observed prior to late June – early July was solely due to *C. partellus* infestation. In the entire study, the highest proportion of dead heart were observed in the second crop of maize and during the tassel+cob stages. The finding of the current study is inline with that of Chabi-Olaye et al. (2006), who investigated the relationship between soil fertility and stem borer damage and yield in insecticide-free maize-based cropping systems in Cameroon and recorded dead heart percentages ranging from 2.90% to 11.20%. They attributed this high proportion of dead heart to *B. fusca*, it was the most dominant speciess in Cameroon at the time. Even under insecticide-treated fields, dead heart is still a common occurrence due to stem borer infestations. Rauf et al. (2017) reported dead heart occurrence in maize fields separately treated with foliar insecticides (chlorantraniliprole, fipronil, spinosad and flubendiamide) and granular insecticides (carbofuran, cartap, and monolmelypo) in Pakistan. Rauf et al. (2017) attributed the dead heart damage to *C. partellus*, which is the dominant species in Pakistan. The proportion of dead heart is reportedly affected by many factors such as maize density, cropping systems, nutrition and principally by pest diversity and abundance (Ogunwolu et al., 1981; Van den Berg and Van Rensburg, 1991; Chabi-Olaye et al., 2006; Rauf et al., 2017).

4.3. Dispersion of *Chilo partellus* and Optimal sampling size of maize

4.3.1. Dispersion (Spatial distribution) of *Chilo partellus* by Taylor Power Law

Taylor Power Law (TPL) was used to determine the spatial distribution of *C. partellus* on first and second crop of maize in 2018 and 2019 at different maize phenology. The maize stages were divided into the vegetative (germination to V8-9), maturity (tassel+cob) and the dryness stages. The Taylor aggregation index (b) for the different stages of *C. partellus* at different maize phenology in the first crop of maize in 2018 is shown in Table 4.3. The b value for the larvae and larvae+pupae stages at all maize phenologies were significantly ($P < 0.05$) gretaer

than 1. The Taylor aggregation index (b) for the second crop of maize in 2018 are presented in Table 4.4. Apart from the larvae and larvae+pupae stages at the vegetative stages of maize, all the b values were greater than 1. In 2019, all Taylor aggregation index (b) for all stages of *C. partellus* in all maize phenologies in the first crop of maize were significantly ($P < 0.05$) greater than 1 (Table 4.5). The Taylor aggregation index (b) for the all stages of *C. partellus* in the second crop of maize of 2019 is presented in Table 4.6. The b values for all larvae and larvae+pupae stages were greater than 1 except for the larvae and larvae+pupae values at the dryness stages of maize.

Table 4.3. Regression statistics and parameter estimates of Taylor's power law of *Chilo partellus* on maize (first crop) in 2018 in Adana, Turkey

		Taylor Power's Law coefficients					ANOVA results		
Insect stage	**Plant stage**	**Log(a)**	**b**	**t**	***p***	**R^2**	**F**	**df**	***p***
larvae	Vegetative	0.845	1.689	12.624	0.006	0.988	159.355	1, 2	0.006
	Maturity	0.444	1.399	8.878	0.0001	0.898	78.813	1, 9	0.0001
	Dryness	0.406	1.344	9.563	0.066	0.989	91.544	1, 1	0.066
Larvae	Vegetative	0.847	1.926	7.340	0.005	0.947	53.872	1, 3	0.005
+	Maturity	0.305	1.411	3.257	0.01	0.541	10.611	1, 9	0.01
pupae	Dryness	0.435	1.435	14.078	0.045	0.995	198.188	1, 1	0.045

Slopes (b) are significantly > 1 (aggregated distribution); student t-test ($P < 0.05$)

Table 4.4. Regression statistics and parameter estimates of Taylor's power law of *Chilo partellus* on maize (second crop) in 2018 in Adana, Turkey

Insect stage	Plant stage	Taylor's Power Law coefficients				R^2	ANOVA results		
		Log(a)	b	t	*p*		F	df	*p*
larvae	Vegetative	0.455	0.479	0.601	0.580	0.083	0.361	1, 4	0.580
	Maturity	0.347	1.317	20.85	0.0001	0.986	434.779	1, 6	0.0001
	Dryness	0.113	1.297	7.124	0.006	0.944	50.748	1, 2	0.006
Larvae + pupae	Vegetative	0.597	-0.181	-0.224	0.834	0.012	0.050	1, 4	0.834
	Maturity	0.407	1.426	12.897	0.0001	0.965	166.342	1, 6	0.0001
	Dryness	0.143	1.154	5.664	0.011	0.914	32.078	1, 3	0.011

Slopes (b) are significantly > 1 (aggregated distribution); student t-test ($P < 0.05$)

Table 4.5. Regression statistics and parameter estimates of Taylor's power law of *Chilo partellus* on maize (first crop) in 2019 in Adana, Turkey

		Taylor Power's Law coefficients					ANOVA results		
Insect stage	**Plant stage**	**Log(a)**	**b**	**t**	***p***	**R^2**	**F**	**df**	***p***
larvae	Vegetative	0.680	1.138	3.015	0.095	0.820	9.093	1, 2	0.095
	Maturity	0.122	1.094	5.085	0.004	0.838	25.852	1, 5	0.004
	Dryness	0.853	1.892	$94x10^6$	0.0001	1.0	$94x10^8$	1, 1	0.0001
Larvae + pupae	Vegetative	0.727	1.183	2.838	0.105	0.801	8.052	1, 2	0.105
	Maturity	0.531	1.462	5.023	0.002	0.808	26.226	1, 6	0.002
	Dryness	1.816	3.309	3.04	0.0001	1.0	6.250	1, 1	0.0001

Slopes (b) are significantly > 1 (aggregated distribution); student t-test ($P < 0.05$)

Table 4.6. Table 4.6. Regression statistics and parameter estimates of Taylor's power law of *Chilo partellus* on maize (second crop) in 2019 in Adana, Turkey

		Taylor's Power Law coefficients					ANOVA results		
Insect stage	**Plant stage**	**Log(a)**	**b**	**t**	***p***	**R^2**	**F**	**df**	***p***
larvae	Vegetative	0.116	1.147	1.147	0.0001	0.397	1.315	1, 3	0.0001
	Maturity	0.940	1.307	13.91	0.001	0.985	193.478	1, 3	0.001
	Dryness	-0.270	0.720	0.459	0.726	0.174	0.210	1, 1	0.726
Larvae + pupae	Vegetative	0.116	1.147	1.147	0.0001	0.397	1.315	1, 3	0.0001
	Maturity	0.759	1.164	4.384	0.022	0.865	19.223	1, 3	0.022
	Dryness	-0.342	0.637	1.155	0.454	0.572	1.335	1, 1	0454

Slopes (b) are significantly > 1 (aggregated distribution); student t-test ($P < 0.05$)

4.3.2. Determination of optimal sample size

The optimum sample units (N) for maize required to obtain different population densities of *C. partellus* within a certain reliability was estimated using coefficients of TPL (Karandinos, 1976). In this study, three precision levels were used: D = 0.01, D = 0.20, and D = 0.30. The mean number of larvae+pupae were used for the estimations at different maize phenologies in 2018 (Figure 4.8) and 2019 (Figure 4.9). For instance, in the vegetative stage of the first crop of maize of 2018, a larvae+pupae density of 1/plant requires 1374, 344 and 153 maize sample units at precision levels of 0.10, 0.20, and 0.30, respectively (Figure 4.8A). At the vegetative stage of the second crop of 2018, a larvae+pupae density of 1/plant required 774, 193, and 86 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively (Figure 4.8B). The maize sampling units for the tassel+cob stage of the first and second crops of maize in 2018 are shown in Figure 4.8C and Figure 4.8D, respectively. For the dryness stage of the first and second crop of maize in 2018, the maize sampling units are presented in Figure 4.8E and Figure 4.8F, respectively.

The optimal sampling size of maize at different densities of *C. partellus* (larvae+pupae) at different maize phenologies for the first and the second crop of maize in 2019 is provided in Figure 4.9. For instance, in the first crop of maize at the vegetative stage, a larvae+pupae mean density of 1/plant requires 1045, 261 and 116 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively (Figre 4.9A). On the other hand, a larvae+pupae density of 1/plant requires 256, 64, and 28 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively at the vegetative stage of the second crop of maize (Figure 4.9B). The maize sampling units for the tassel+cob stage of the first and second crops of maize in 2019 are shown in Figure 4.9C and Figure 4.9D, respectively. For the dryness stage of the first and second crop of maize in 2019, the maize sampling units are presented in Figure 4.9E and Figure 4.9F, respectively.

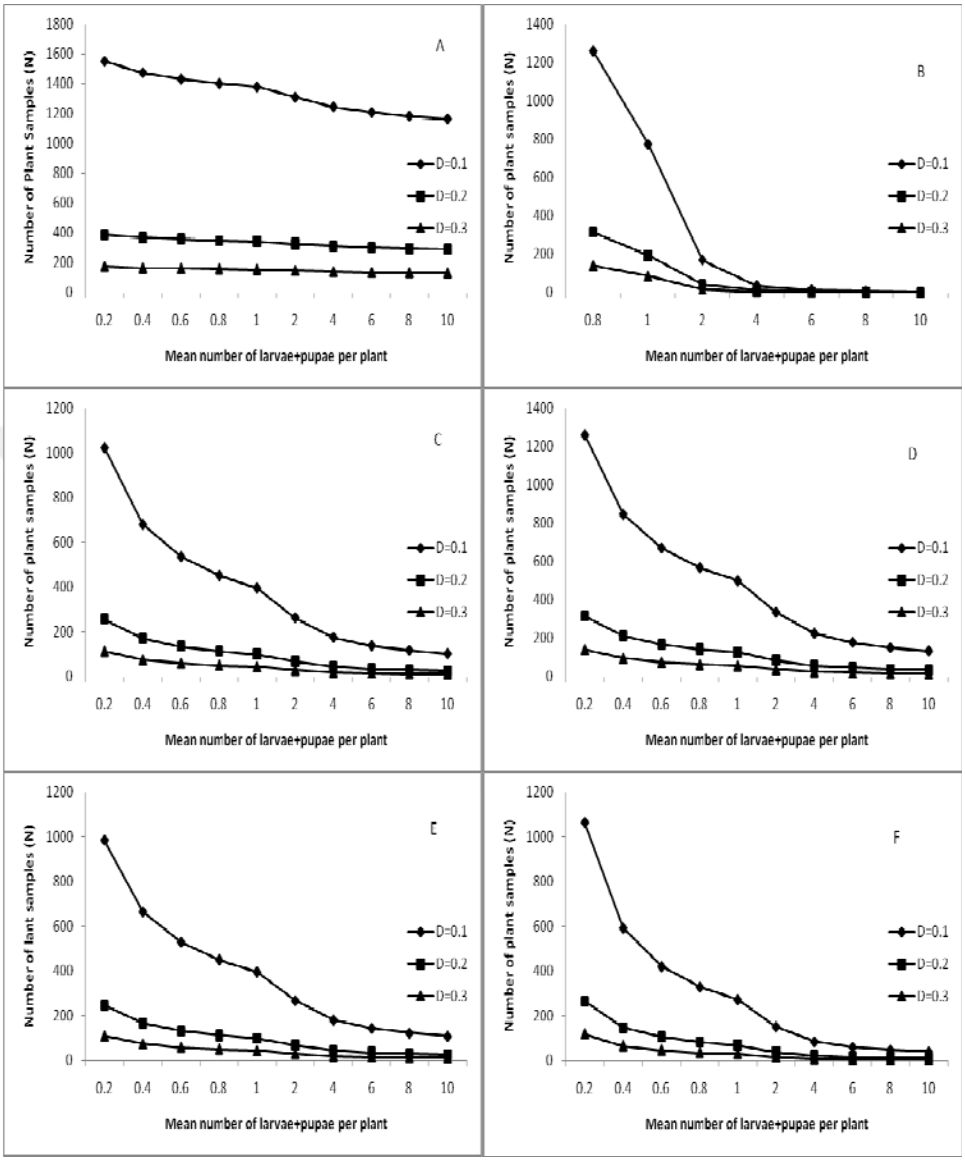


Figure 4.8. Optimal sample size (N) for maize infested by *Chilo partellus* larvae+pupae for 2018. A, C and E for vegetative, maturity and dryness stages - first crop. B, D and F for vegetative, maturity and dryness stages - second crop

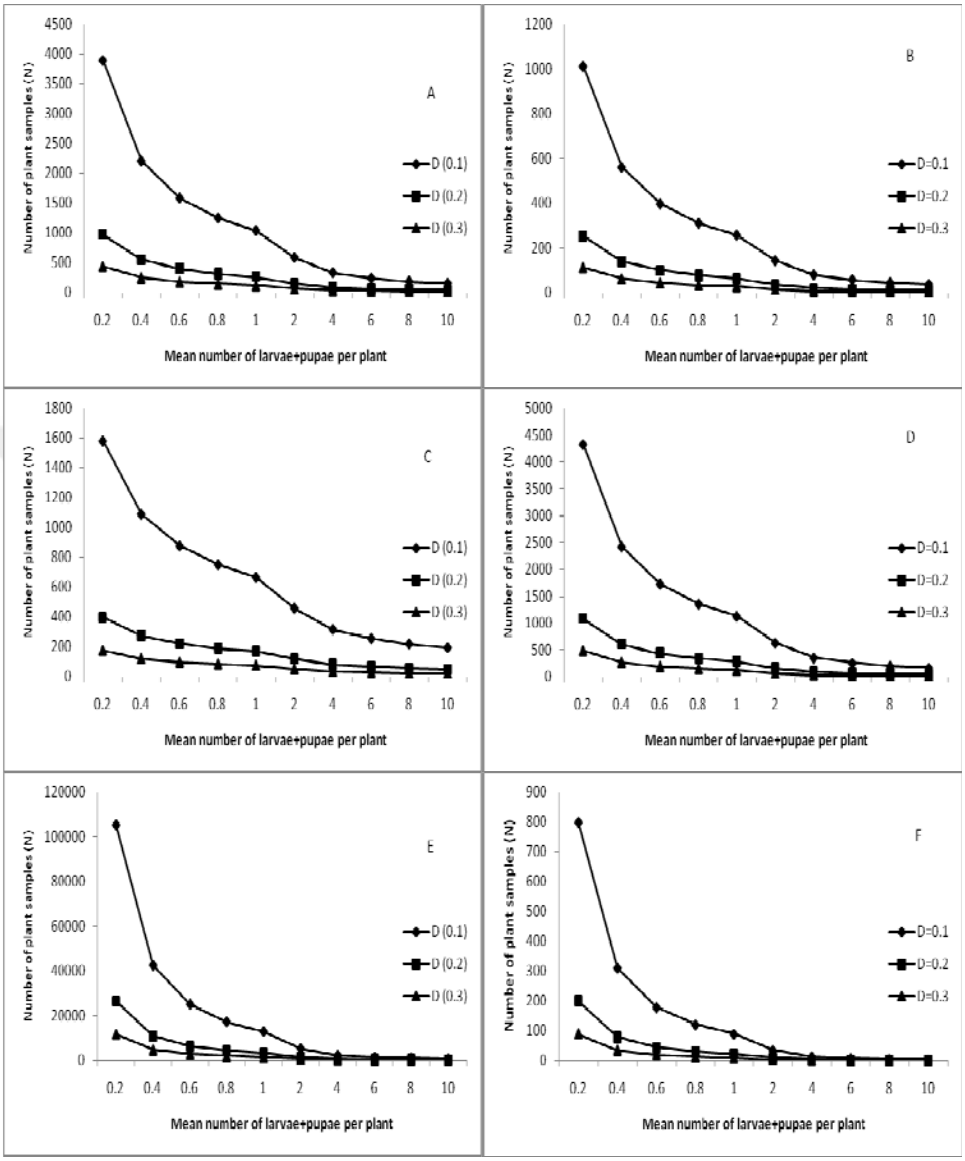


Figure 4.9. Optimal sample size (N) for maize infested by *Chilo partellus* larvae+pupae for 2019. A, C and E for vegetative, maturity and dryness stages - first crop. B, D and F for vegetative, maturity and dryness stages - second crop

In general, the Taylor aggregation index (b) of *C. partellus* at all stages of maize was greater than 1 – indicating that *C. partellus* exhibits an aggregated (clumsy) spatial distribution. This aggregated spatial configuration of *C. partellus* seems to be common with many lepidopteran pests. Silva et al. (2018) reported an aggregated spatial distribution for the bollworm *Helicoverpa* sp. (Lepidoptera: Noctuidae) and the fall armyworm caterpillars *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) larvae on Bt and non-Bt maize. In the United States of America, Nault and Kennedy (1996) also reported that *O. nubilalis* larvae exhibits an aggregated spatial distribution. Hunter (2000) and Coster-Longman et al. (2002) suggested many reasons why insect utilizes an aggregated spatial distribution, amongst which are the ease of finding mates, breeding sites and finding food, protection against harsh climatic conditions, biological control agents and insecticides.

One tenet of IPM is to understand the spatial distribution (dispersion) of any pest (Ferguson et al., 2003; Duarte et al., 2015). Taylor's Power Law regression has been extensively used in ecological and entomological studies to ascertain the dispersion of many insect pests (Atakan, 2010; Arruda-Neto et al., 2014; Duarte et al., 2015; Damos, 2018).

The development of sampling is strongly influenced by the dispersion of the insect. Consequently, many sampling models have been developed based on parameters of TPL such as Karandinos (1976) optimal sampling plant. As expected, in the current study, the optimum sample size increased with increased precision and low pest density, as supported by Karandinos (1976), Trumper and Garat (2001) and Lessio and Alma (2006). In most cases, the optimum sample size reduced about 3-4 folds as the precision level increased from 0.10 to 0.20 for the same population mean density, indicating that optimal sample size is strongly dependent on precision level. For the purpose of IPM purposes, a lower number of sample units are required to reach a desired outcome; therefore, it is ideal to use precision levels of 0.20 and 0.30 for the determination of optimal sampling size.

According to Trumper and Garat (2001), adapting precision levels of 0.20 is realistic for pest management decision making. In the current study, the sampling plan was estimated considering the stage of the pest and the phenology of the plant. This approach is highly supported by Manly (1989) and Trumper and Garat (2001), who posited that in order to fully understand the dynamics of any pest, these variables must be taken into account, contrary to just studying the pest over the entire crop season regardless of plant phenology and pest developmental stage.

4.4. Parasitism of *Chilo partellus* eggs in field conditions and morphological identification of the egg parasitoid

4.4.1. Parasitism of *Chilo partellus* eggs in field conditions

The rate of natural parasitism (%) of field-collected egg masses of *C. partellus* by *Trichogramma evanescens* in 2018 and 2019 are shown in Table 4.7 and Table 4.8, respectively. Preliminary studies conducted in 2017 showed that it gets easier finding parasitized egg masses of *C. partellus* in August and September. Thus, much emphasis in this study was during this period for 2018 and 2019.

In 2018, the first parasitized egg masses were observed on 09 August with a parasitism rate of 71.43% (Table 4.7). Parasitism rates reached 100% on 16 August and 11 September. Three parasitism rates of 0.0% were recorded on 02 August, 23 August and 06 September. On the other hand, the first parasitism was observed on 31 July in 2019 at a rate of 20.0% (Table 4.8). The rate of parasitism in 2019 ranged between 0.0% to 50.0%. The highest parasitism rate (50.0%) was observed on 18 September, followed by a parasitism rate of 40.0% on 15 August and 04 September.

Table 4.7. Percentage of natural parasitism of eggs masses of *Chilo partellus* by *Trichogramma evanescens* on second crop of maize in Balcalı, Adana, 2018

Date	Number of egg masses collected	Number of egg masses parasitized	Percentage of parasitism
02 - August	4	0	0
09 - August	14	10	71.43
16 - August	6	6	100
23 - August	11	0	0
31 - August	8	6	75.0
06 - September	12	0	0
11 - September	10	10	100

Table 4.8. Percentage of natural parasitism of eggs masses of *Chilo partellus* by *Trichogramma evanescens* on second crop of maize in Balcalı, Adana, 2019

Date	Number of egg masses collected	Number of egg masses parasitized	Percentage of parasitism
24 - July	1	0	0
31 - July	10	2	20
07 - August	8	3	37.5
15 - August	10	4	40
21 - August	15	3	20
28 - August	20	6	30
04 - September	25	10	40
11 - September	22	8	36.36
18 – September	6	3	50
25 - September	8	2	25

4.4.2. Morphological identification of egg parasitoid of Chilo partellus, Trichogramma evanescens

The parasitoid species of this study was identified on the base of morphological study of male genitalia and male antennae (Nagarkatti and Nagaraja, 1977; Pinto, 1999). Morphological keys of Pintureau (2008) and Sorokina (1993) were used for the identification. The parasitoid species was identified as *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae).

The species *T. evanescens* can be distinguished from other species by the presence of long setae on male antennae. Length of setae in 2.5-3.0 mm or more times longer than the width of clava. Male genitalia of *T. evanescens* represent a well-visible wide tip and wide lateral lobes of dorsal extension of phallobase of genitalia and a long, narrow and sharp intervorsellar process, or ventral extension of phallobase. These morphological details of the male antennae and genitalia of *T. evanescens* are shown in Figure 4.10.

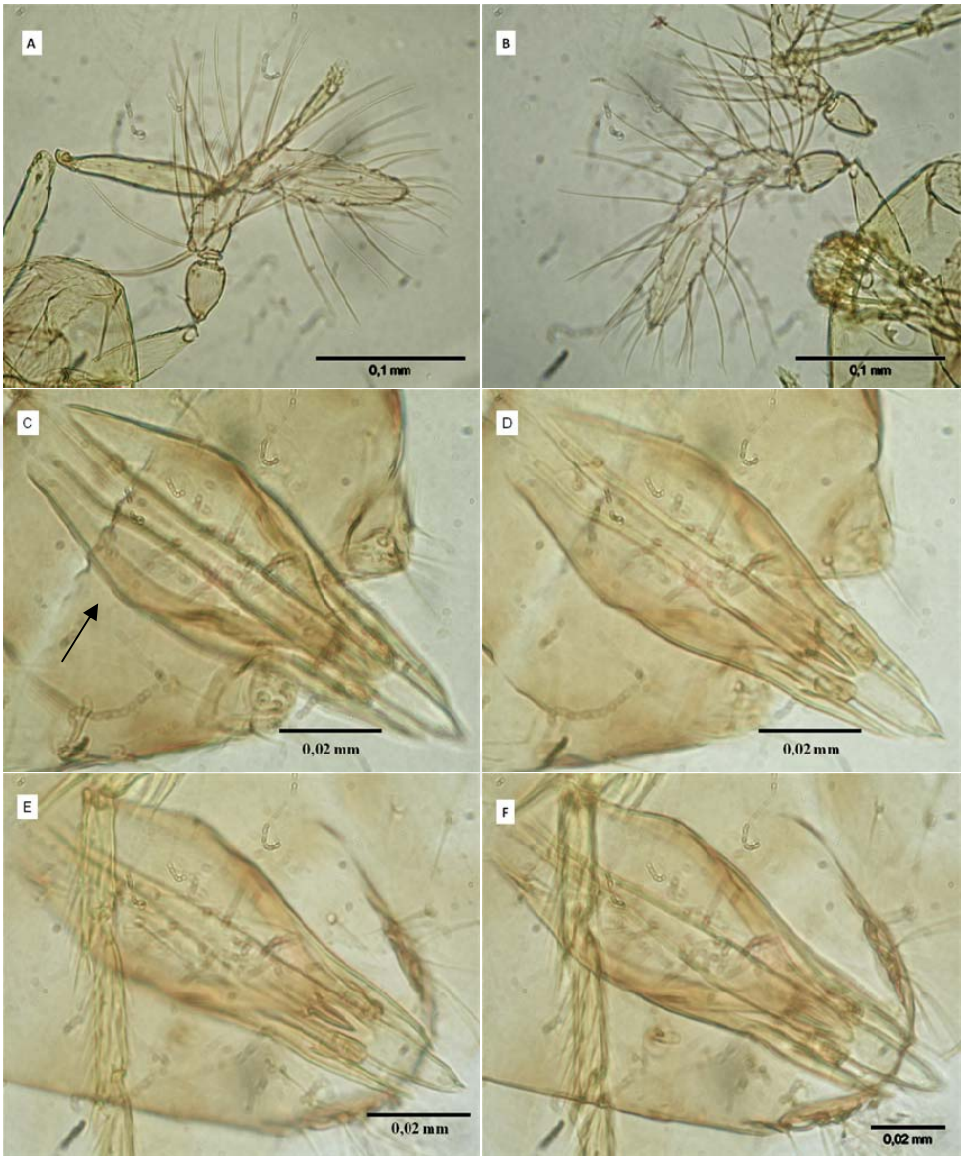


Figure 4.10. Morphological characteristics of male *Trichogramma evanescens* reared from eggs of *Chilo partellus* in Adana, Turkey. A – length of setae in 2.5-3.0 mm and more times, longer than the width of clava, B – length of setae in 3.0 and more times longer than the width of setae, C – wide tip and wide lateral lobes of dorsal extension of phallobase, D – ventral extension of phallobase, E – wide tip and wide lateral lobes of dorsal phallobase enlarged, F – ventral extension of phallobase enlarged (@ Dr. Victor Fursov)

According to the original results, it may be suggest that recorded levels of parasitism of *T. evanescens* in the egg masses of *C. partellus* can represent a significant role of this parasitoid as a natural biological control agent in the suppression of the population of *C. partellus* in the experimental field. It should be mentioned that the high parasitism recorded represent egg masses that parasitoids emerged from and not the actual number of eggs per egg mass. In Turkey, *T. brassicae* and *T. evanescens* have been used against lepidopterous pests. *Trichogramma brassicae* Bezdenko was used in maize fields in Duzce Province in Turkey to achieve almost total control (90.0% parasitism two years after initial release) of *O. nubilalis* Hübner (Noctuidae) (Kutuk, 2017). Öztemiz et al. (2017) reported that *T. evanescens* reduced *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) of apple fruit to 9.66%, compared to 34.0% in control plots in Pozantı, Turkey. Low to medium percentages of parasitism (19.0 – 59.12%) of *T. evanescens* on eggs of *Sesamia nonagrioides* have been recorded in life table experiments in Adana, Turkey (Sertkaya et al.,1999; Sertkaya and Kornoşor, 2002). Kayapınar and Kornoşor (1992) and Özpınar and Kornoşor (1994) also recorded very high parasitism of *O.nubilalis* in maize fields by *T. evanescens* in many parts of Turkey. It is known that excessive use of insecticides is not only dangerous for the environment but it is also costly (Achiri et al., 2016). The use of alternative methods such as biological control of *C. partellus* is highly investigated in many parts of the world (Jalali and Singh, 2006; Shera et al., 2017; Thubru et al., 2018). The first parasitized eggs of *C. partellus* in maize agro-ecosystem of Turkey were recorded by Can Cengiz et al. (2016). Can Cengiz et al. (2016) recorded *Telenomus busseolae* Gahan (Hymenoptera: Platygastridae) and *T. brassicae* as egg parasitoids of *C. partellus* in Hatay Province, Turkey.

This first report of *T. evanescens* in this study and the previous report of Can Cengiz et al. (2016) are supportive of adaptive local biological control of stem borers including *C. partellus*. In the findings of Can Cengiz et al. (2016), parasitism of *C. partellus* was recorded mostly in the second crop probably

emphasizing the general parasitoid nature of the parasitoids which are often in the field in the second crop of maize alongside high populations of native maize stem borers.

Morphological parameters are successfully being used to identify many insect species. The male genitalia is a very vital diagnostic character of the *Trichogramma* genus (Nagarkatti and Nagaraja, 1971). Pinto (1999) established very concise procedures, terminologies and abbreviations of morphological characters and ratios of male antennae and genitalia of *Trichogramma* genus. Some of these characters include: length of aedegus (AL), length of basal part of aedegus (AL-B), length of dorsal aperture (DA-L), width of dorsal lamina (DLA), length of genital capsule (GC), length of longest seta of flagellum (SL), maximum width of flagellum (FW) and more (Woelke et al., 2019). There are many citations of successful identification of *Trichogramma* spp. using morphological characters. Birova and Kazimirova (1997) used these male genitalia and antenna characters to identify *Trichogramma danubiense* sp. n. (Hymenoptera: Trichogrammatidae). Woelke et al. (2019) used morphological characters of male genitalia and antennae to describe two new *Trichogramma* species. Sorokina (1993) and Pintureau (2008) used these characters and identified *T. evanescens* and *T. brassicae*. It is considered that the species *T. brassicae* as morphologically closest species to *T. evanescens* which has minor morphological differences (Pintureau, 2008).

4.5. Degree Days (DD) Models

4.5.1. Population fluctuations of *Chilo partellus* and environmental factors (Temperature and Relative Humidity)

The interplay of temperature and relative humidity on the population dynamics of *C. partellus* is shown in Figure 4.11 for 2017, 2018, and 2019. The temperature during the study period ranged from 20.25 – 30.88 °C, 19.51 – 29.87 °C, and 19.63 – 29.75 °C in 2017, 2018, and 2019, respectively. The degree-days (DD) followed the same pattern as the weekly mean temperature. The weekly mean temperature was not significantly different ($\chi^2 = 0.168$, df = 1, $p = 0.682$) for 2017 and 2018. The relative humidity (RH%) during the study period ranged from 55.0 – 67.15 RH%, 24.51 – 67.38 RH%, and 48.69 – 71.5 RH% in 2017, 2018, and 2019, respectively. In 2017, the first and second peaks were observed on 11 May and 20 July, respectively. The first and second peaks occurred on 21 May and 02 August in 2018. In 2018, the major peak was observed in the first crop about 5-11 June. Two small peaks were observed in the mixed and second crop just about 22 July – 07 August. Since both small peaks occurred at a short time interval, they were indicative of members of the same generation. In 2019, the first and second peaks were observed on 31 May and 02 August. The total number of larvae collected in 2017 was significantly higher ($\chi^2 = 33.989$, df = 1, $p = 0.001$) than that of 2018 for the same period of the year. In General, regression analysis revealed that the regression between temperature and mean *C. partellus*, and humidity and mean *C. partellus* larvae were not significant for the first and second crop of maize in the entire study period (Table 4.9).

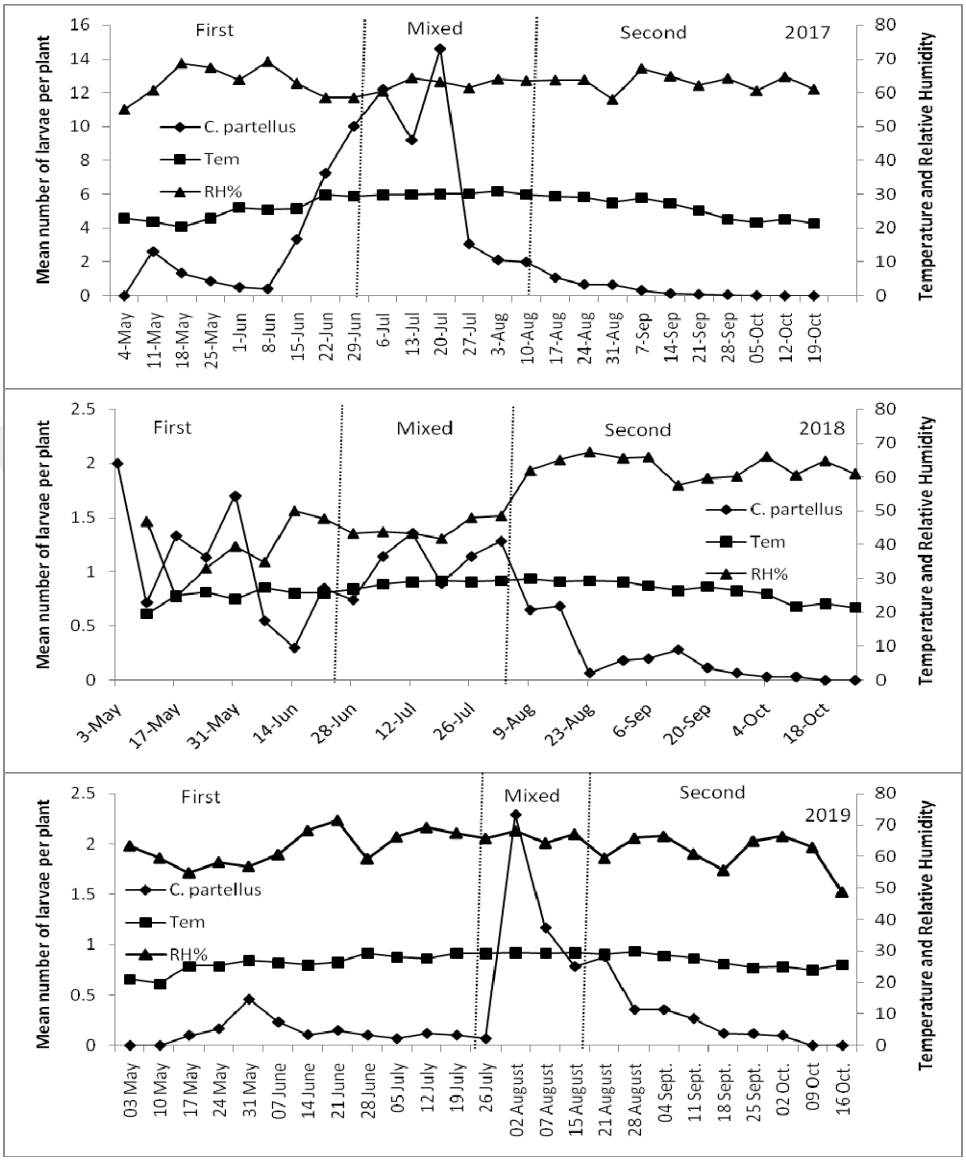


Figure 4.11. Population dynamics of *Chilo partellus* in two maize growing seasons in 2017, 2018 and 2019 and environmental parameters (temperature and relative humidity). first – first maize growing, season – second maize growing season, and mixed – represent a month period within which both first and second crops were on the field

Table 4.9. Regression of *Chilo partellus* larvae with environmental parameters (temperature and relative humidities) according to maize growing seasons and years

		Temperature			Humidity		
Year	**Maize season**	**Regression equation**	**R^2**	***P* - value**	**Regression equation**	**R^2**	***P* - value**
2017	First crop	y = 310.277-11.06*x*	0.435	.330	y = 150.958-1.470*x*	0.123	.793
	Second crop	y = 115.45*x*-3023.33	0.589	.107	y = 889.77-8.67*x*	0.180	.805
2018	First crop	y = 257.702-7.802*x*	0.665	.013	y = 24.588-0.046*x*	0.121	.693
	Second crop	y = 0.412*x*-9.584	0.444	.111	y = 6.720-0.082*x*	0.619	.018
2019	First crop	y = 0.016*x*-0.272	0.417	.138	y = 0.439-0.005*x*	0.219	.452
	Second crop	y = 0.186*x*-4.599	0.519	.102	y = 0.054*x*-2.859	0.307	.359

4.5.2. Proportion (%) of cumulative *Chilo partellus* larvae per maize plant

The proportion (%) of cumulative larvae numbers of *C. partellus* for 2017, 2018, pooled (2017 and 2018), and 2019 data against cumulative degree-days (DD) is given in Figure 4.12. The first peak occurred on 11 May and 21 May in 2017 and 2018, respectively, corresponding to the accumulation of 12 and 24 degree-days, respectively. Both the 2017 and 2018 first peaks ended on the same day, 25 June, corresponding to the accumulation of 98 degree-days. The first peak of 2019 followed very similar pattern as in 2017 and 2018, however ending on 28 June, corresponding to the accumulation of 135 degree-days. The second peak in 2017 and 2018 started on the same week, (~ 5 July), corresponding to the accumulation of 116 degree-days. The second peak ended on 05 and 12 October in 2017 and 2018, representing an accumulation of 372 and 387 degree-days, respectively. On the other hand, the second peak for 2019 started rather later, 13 July, and ended on 19 October, representing an accumulation of 153 and 401, respectively.

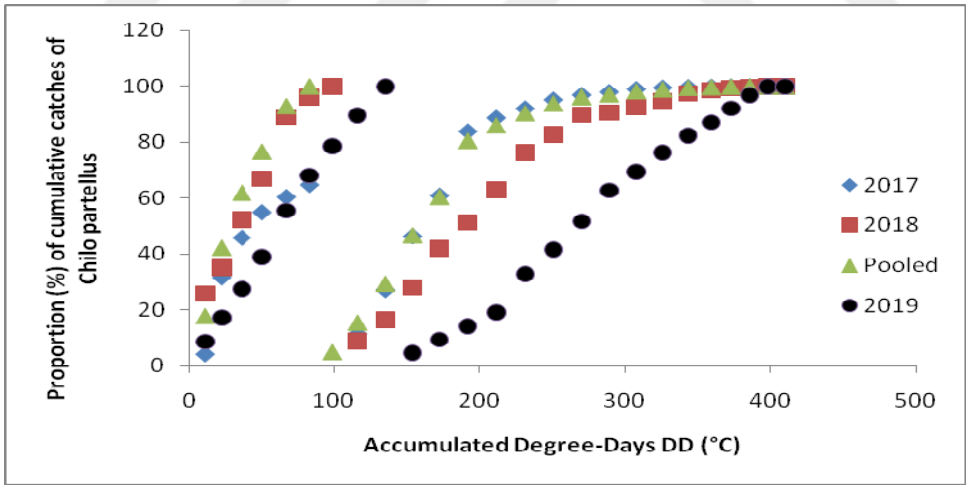


Figure 4.12. Proportion of cumulative *Chilo partellus* larval count and cumulative degree-days during the first and second crop in 2017 - 2019 and pooled data in maize field. Pooled data was obtained from 2018 and 2019.

4.5.3. Parameters of the forecasting model

The 3-parameter 'Boltzman' type and the 4-parameter logistic regression models parameters for estimating the time and the peak periods of *C. partellus* in maize fields are given in Table 4.10. The parameter c of the 'Boltzman' type and the logistic regression model represented 50% of the cumulative *C. partellus* numbers for each season. The parameter c was 60 DD and 65 DD for peak 1 from the 'Boltzman' and logistic regression models, respectively. For the first peak, the R^2 values were 0.769 and 0.886 for 'Boltzman' and logistic regression models, respectively. For the second peak, the parameter c was 264 DD and 278 DD for 'Boltzman' type and logistic regresion models, respectively. The R^2 value for the second peaks were 0.754 and 0.743 for 'Boltzman' type and logistic regressions, respectively. The estimated parameters of the 3-parameter 'Boltzman' type and logistic regression model functions are summarized in Table 4.10.

Table 4.10. Parameter estimates and coefficient of determinations of two non-linear models: 3-parameter 'Boltzman' type and 4-parameter logistic regression, in describing *Chilo partellus* phenology

Peaks	**Parameter estimates**			
	'Boltzman' type function		**Logistic regression function**	
Peak 1	a	1.133	a	-93.343
	b	0.4	b	-0.269
	c	61	c	65
	-		d	93.494
	R^2	0.769	R^2	0.886
Peak 2	a	2.135	a	6.992
	b	0.01	b	-2.061
	c	264	c	278
	-		d	-5.554
	R^2	0.754	R^2	0.743

Parameters were estimated from the pooled data in order to get a general performance of the models. a and b are the lower and upper asymptote of the regression function curve. c is the cummulative DD at which 50% of the moth is caught per generation. d is a contant adjusting the shape of the curve

4.5.4. Statistical evaluation of regression models

The observed and predicted regression statistics for the 3-parameter 'Boltzman' type and 4-parameter logistic regression models using the data of 2019 are summarized in Table 4.11. The R^2 values for all models and all peaks were greater than 0.9. The corrected Akaike Information Criterion (AICc) for 'Boltzman' type and logistic regression models were 30.122 and 34.396, respectively for the first peak. The AICc values for 'Boltzman' type and logistic regression models were 17.167 and 56.341, respectively for the second peak. For both peaks, the 'Boltzman' type regression model had a lower Akaike delta score (ΔAICc) than the logistic regression model; consequently a lower Akaike weight (Wi).

Table 4.11. Regression statistics for predicted versus observed percentage, and statistical classification criteria of two non-linear regression models: 3-parameter 'Boltzman' type and 4-parameter logistic regression in describing *Chilo partellus* phenology

		Regression characteristics				**Statistical information criterion**				
Peak	**Model**	**n**	**Regression equation**	R^2	**Adj R^2**	**F**	**AIC**	**AICc**	**ΔAICc**	***W*i**
1	Boltzman	9	y = 10.67 + 0.726*x*	0.980	0.977	336.181	30.122	**34.922**	**0**	**<.001**
	Logistic	9	y = 19.293 + 0.674*x*	0.963	0.957	179.952	34.396	44.396	9.474	1
2	Boltzman	16	y = 0.349*x* – 48.44	0.997	0.987	5089.15	17.167	**19.167**	**0**	**<.001**
	Logistic	16	y = 0.320*x* – 25.65	0.964	0.961	371.039	56.341	59.981	40.814	1

n – number of samples, Adj R^2 – Adjusted *R* square, AIC – Akaike's Information Criterion, AICc – corrected Akaike's Information Criterion, ΔAIC – AIC delta scores (relative difference between the best model), *W*i – Akaike weights (probability of the best model). The values in bold indicate better model performance with regard to the applied statistical criterion used.

4.5.5. Regression functions to predict seasonal peaks

The mean numbers of *C. partellus* of the first and second peaks of 2019 were fitted into a plot using the 3-parameter 'Boltzman' type and and the 4-parameter logistic regression functions as shown in Figure 4.13. Figure 4.13a shows the first peak observed and the first peak predicted by the 'Boltzman' type regression. A strong Pearson product-moment coefficient (r) was observed (r^2 = 0.990, n = 9, p = 0.0001) between the observed and the predicted peaks. The second peak observed and the second peak predicted by 'Boltzman' type regression model is presented in Figure 4.13b. A strong positive correlation was observed (r^2 = 0.973, n = 16, p = 0.0001) between the observed and the predicted peaks. The first peak observed and the first peak predicted by the logistic regression function is shown in Figure 4.13c. A strong positive correlation was observed (r^2 = 0.976, n = 9, p = 0.0001). A strong positive correlation (r^2 = 0.973, n = 16, p = 0.0001) was also recorded between the second peak observed and the second peak predicted by the logistic regression function (Figure 4.13d)

The parameters of the 'Boltzman' type and the logistic regression equation from the pooled data were used to fit a curve for all the data collected in the study (Table 4.12). There were good fits between observed and predicted data for the first and second peaks for both models. The R^2 value were greater than 0.80 for all peaks except for the second peaks in 2017 irrespective of the model used.

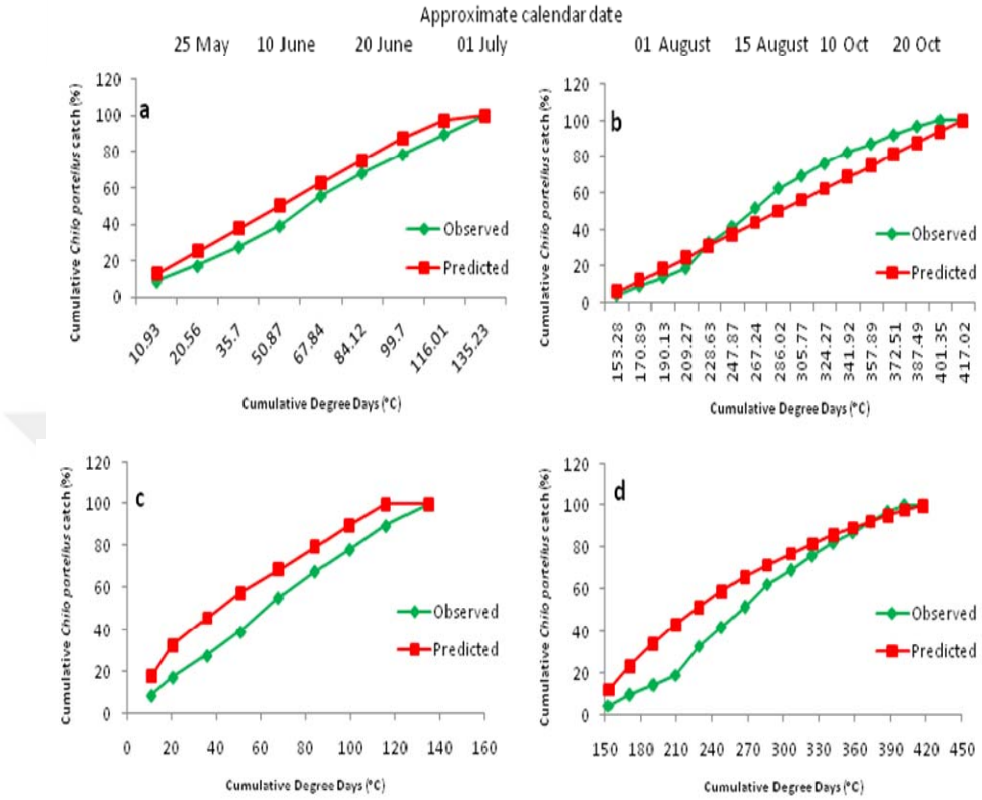


Figure 4.13. Observed and predicted cumulative *Chilo partellus* numbers relative to cumulative degree-days (DD) using 2019. data according to the 3-parameter 'Boltzman' type and logistic regression function in maize fields in Adana, Turkey. a – first peak predicted by 'Boltzman' type regression, b – second peak predicted by 'Boltzman' type regression, c – first peak predicted by logistic regression function, d – second peak predicted by logistic regression function.

Table 4.12. Estimated regression equations for all years of *Chilo partellus* phenology, and observed and predicted times of 50% (DD50) *C. partellus* peak emergence relative to degree-days using the two nonlinear models; 3-parameter 'Boltzman' type and 4-parameter logistic regression function

Peak	Year	n	model	Regression equation	R^2	Adj. R^2	AIC	DD_{50} Observed	DD_{50} Predicted
1	2017	7	Boltzman	$y = 0.532x + 2.050$	0.912	0.894	33.376	90.13	80.50
		7	Logistic	$y = 0.532x + 2.050$	0.912	0.894	33.376	90.13	90.13
	2018	7	Boltzman	$y = 0.551x + 15.078$	0.967	0.960	26.656	63.38	81.06
		7	Logistic	$y = 0.551x + 15.084$	0.967	0.960	26.652	63.37	62.51
	2019	9	Boltzman	$y = 10.67 + 0.726x$	0.980	0.977	30.122	64.01	54.19
		9	Logistic	$y = 19.293 + 0.674x$	0.963	0.957	34.396	64.01	45.56
2	2017	16	Boltzman	$y = 0.179x + 7.191$	0.707	0.686	90.781	239.16	375.37
		16	Logistic	$y = 0.179x + 7.193$	0.707	0.686	90.78	239.15	362.76
	2018	17	Boltzman	$y = 0.208x - 16.593$	0.878	0.870	84.436	320.16	413.03
		17	Logistic	$y = 0.208x - 16.587$	0.878	0.870	84.441	320.13	337.18
	2019	16	Boltzman	$y = 0.349x - 48.44$	0.997	0.987	17.167	269.635	289.635
		16	Logistic	$y = 0.320x - 25.65$	0.964	0.961	56.341	269.635	252.167

n – number of samples, Adj R^2 – Adjusted R square, AIC – Akaike's Information Criterion, DD_{50} – cumulative degree-days at which 50% of *Chilo partellus* emerged per peak. Regression equation, R^2, Adj R^2, AIC and DD_{50} were calculated from observed data. DD_{50} was obtained from data predicted by the respective nonlinear regression functions.

Larval/plant counts have been successfully used for surveillance and predictions of insect phenology (Manyangarirwa et al., 2003, Marchioro and Foerster. 2011; Mosiane et al., 2003). This study is the first to characterize the fluctuation of *C. partellus* vis-à-vis degreee-days data using *in situ* observations in Turkey. Studies as this are crucial since insect pest and natural enemies often show varying levels of physiological and behavioural plasticity to temperature changes (Mutamiswa et al., 2017).

Chilo partellus shows two distinct peaks with 1-2 (insignificant) peaks. Each maize growing season had a *C. partellus* peak. The second peak occurred in the period (mixed crop), when both first and second maize crops were on the field. For both years, there was a small (insignificant) peak within a very short time interval to the second peak. According to Damos and Savopoulou-Soultani (2010), binomal peaks occurring at very short time interval should be considered as members of the same generation. In 2017, the first peak was a minor peak while the second was a major peak. This minor-major peak distinction was not easy to make for 2018 owing to the very subtle differences between first and second peak larval counts . The first maize crop in the Mediterranean belt of Turkey is planted in March, however, the first record of *C. partellus* was made in May, at a time corresponding to an accumulated degree-days of 21 with 1 May as the biofix.

It is important to note that *C. partellus* starts reducing, and progress consistently, on 6-12 August (13 weeks ~ 344 cumulated DD), at which time, the population of the two major indigenous maize stem borers, *O. nubilalis* and *S. nonagrioides* starts to rise (unpublished data). It is reported that these two indigenous maize stem borers are a major problem for maize in the second crop in Turkey (Okyar and Kornoşor, 1997). The results revealed that although it is widely reported that temperature largely plays a role in the phenology of ectotherms (e.g insects), the relationship is not necessarily a simple linear one as is the case with *C. partellus*. Furthermore, polynomial functions (quadratic, cubic, fourth-sixth power) were also evaluated to established a relationship between temperature and

C. partellus phenology, and none was able to predict the phenology to an acceptable degree in this study. Nevertheless, Akotsen-Mensah et al. (2011) reported that a sixth-ordered polynomial had a high predictive potential of the flight activity of plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) in peach orchards in Alabama, the United States of America.

Nonlinear regression and degree-days (DD) models have been widely used to predict and monitor biological events (emergence, spatio-temporal distribution, flight activity, egg laying, egg hatching, larval number) of lepidopterous insects (Damos and Savopouou-Soultani. 2010; Pehlevan and Kovancı. 2017), providing information relevant for sound IPM decision-making. Determination of the first and subsequent peak population of any pest is very important for pest management decision-making. As such a reliable model for predicting insect phenology is very crucial. The parameter c and the R^2 values in the 'Boltzman' type and logistic regression were very similar in this study. This suggest that the two models can accurately predict the phenology of *C. partellus* on maize. Even though the values of the parameter c and R^2 for the 'Boltzman' type and the logistic regression functions were very similar, the statistical evaluation of the regression models revealed that the 'Boltzman' type regression model was a better model, indicated by a lower corrected Akaike Information Criterion (AICc), Akaike delta scores (ΔAICc), and Akaike weights (*W*i) values.

These models were tested on their predictability of the degree-days (DD_{50}) 50% cumulated emergence of *C. partellus*. The values from each model were very similar. The prediction capability of both models was very high as the adjusted coefficient of determination (Adj. R^2) was greater than 0.80 in most cases (Damos and Savopoulou-Soultani, 2010). The models did not accurately predict (Adj. $R^2 <$ 0.80) DD_{50} for the second peak in 2017. These variations are not surprising since sample estimates of population sizes often show inherent biases. Nevertheless, irrespective of the errors and other challenges that resulted into these deviations in

the model's predictive abilities for the second peak in 2017, the peak populations predicted by the models are still within the acceptable limits.

4.6. Evaluation of some biological parameters of *Trichogramma evanescens* under three constant temperatures

4.6.1. Fecundity of *Trichogramma evanescens* on eggs of *Ephestia kuehniella* at three constant temperatures

The fecundity of *T. evanescens* on eggs of *E. kuehniella* at three constant temperatures is presented in Table 4.13. More eggs were laid in 25 °C than the others. The mean number of eggs laid per day was not significantly different ($F = 0.661$, $df = 2, 42$, $P = 0.522$). However, the mean number of eggs laid per female was significantly different ($F = 52.268$, $df = 2, 6$, $P < 0.0001$). The highest mean number of eggs laid per female was 43.87 from 25 °C, followed by 41.80 and 28.13 from 20 °C and 30 °C, respectively (Table 4.13).

The mean numbers of eggs laid per female per day varied in the three constant temperatures (Figure 4.14). In day 1, the number of eggs in 25 °C was significantly higher than those from 20 °C and 30 °C ($F = 64.466$, $df = 2, 42$, $P = 0.0001$). In day 2, the mean number of eggs laid in 20 °C and 30 °C were significantly higher than that in 30 °C ($F = 17.630$, $df = 2, 42$, $P = 0.0001$). No significant differences in the number of eggs laid was observed in the three constant temperatures in day 3 ($F = 1.089$, $df = 2, 42$, $P = 1.09$).

Table 4.13. Fecundity of *Trichogramma evanescens* on *Ephestia kuehniella* eggs in three constant temperatures

Temperature (± 1 °C)	n	Total number of eggs laid by parasitoid female	Mean (± s.e) number of eggs laid/day	Mean (± s.e) number of eggs laid/female
20	15	627	20.90 ± 0.53a	41.80 ± 1.22a
25	15	658	20.91 ± 0.87a	43.87 ± 1.47a
30	15	422	19.37 ± 1.59a	28.13 ± 0.74b

Means in the same column with the same letter(s) are not significantly different (Tukey HSD, $P < 0.05$). n – number of replications; s.e – standard errors of the means.

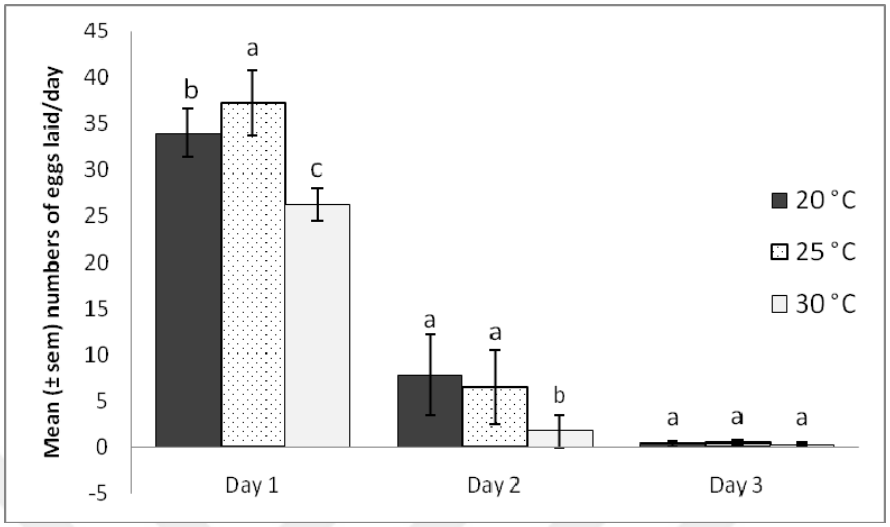


Figure 4.14. Mean numbers of eggs laid by *Trichogramma evanenscens* per day on eggs of *Ephestia kuehniella* in three constant temperatures. Means in the same day with the same letter(s) are not significantly different (Tukey HSD, $P < 0.05$). sem – standard errors of the means.

4.6.2. Fertility of *Trichogramma evanescens* on on eggs of *Ephestia kuehniella* at three constant temperatures

Fertility results of *T. evanescens* on three constant temperatures is presented in Table 4.14. The number of larvae that hatched varied from one temperature to another. The highest total number of *T. evanescens* larvae that hatched from the eggs of *E. kuehniella* was 680 from 25 °C, followed by 568 and 364 from 20 °C and 30 °C, respectively.

The percentage number of larvae that hatched from the prey eggs was significantly different ($F = 12.673$, $df = 2, 42$, $P < 0.0001$). The highest mean percentage of hatched larvae was 92.43%, at 25 °C. The percentage number of hatched larvae from eggs of *E. kuehniella* was greater than 80% for all three temperatures (Table 4.14).

Table 4.14. Fertility of *Trichogramma evanescens* on eggs of *Ephestia kuehniella* at three constant temperatures

Temperature (± 1 °C)	n	Number of hatched larvae of parasitoid	Mean percentage of unhatched larvae of parasitoid (± s.e)	Mean percentage of hatched larvae of parasitoid (± s.e)
20	15	568	13.78 ± 1.13b	86.22 ± 1.13b
25	15	580	7.57 ± 0.63a	92.43 ± 0.63a
30	15	364	13.72 ± 1.15b	86.28 ± 1.15b

Means in the same column with the same letter(s) are not significantly different (Tukey HSD, $P < 0.05$). n – number of replications; s.e – standard errors of the means.

It is widely reported that temperature is one of the most influential factor on the life history parameters of parasitoids (Bazzochi et al., 2003; Li and Mills, 2004; Yilmaz et al., 2007). In this study, there was no differences in the mean number of eggs laid per female for the temperatures tested. However, the mean number of eggs laid per female was significantly higher from 25 °C. In a similar research, Mohammad et al. (2015) investigated the influence of 7 constant temperatures (15, 18, 20, 25, 28 and 30 °C) on some biological parameters of *T. evanescens* on the lesser date moth (LDM) *Bactrachedra amydraula* Meyrick (Lepidoptera: Bactrachedridae), and reported that the highest parasitism by *T. evanescens* was observed from 25 °C, followed by 28 °C, 20 °C, 18 °C, 30 °C, 15 °C and 35 °C. Even though the host (prey) used in the study of Mohammad et al. (2015) was different from that used in current study, the associated results of this thesis are in accordance with the findings of Mohammad et al. (2015). Many *Trichogramma* species tend to have high fecundity rates (parasitism) at temperatures of 25 ± 2 °C irrespective of the host (Maceda et al., 2003; Mawela et al., 2013; Mohammad et al., 2015). Nevertheless, Firake and Khan (2014), reported a steady decrease of fecundity as temperature increased from 20 °C to 35 °C for *Trichogramma poliae* Nagaraja (Hymenoptera: Trchogrammatidae) and *T. chilonis*. The latter is a native natural enemy of *C. partellus*, on eggs masses of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae).

The mean numbers of eggs laid per female per day was affacted by temperature. The highest numbers of eggs laid on the first day was in 25 °C, followed by 20 °C and in 30 °C. On the second day, the number of eggs laid in all temperatures reduced by about 3-4 folds compared to the first day. Very small number of eggs were laid on the third day. The mean number of eggs laid per female generally decreases over time or with age of parasitoid (Reznik et al., 2009). Mansour (2019) investigated the effects of temperature on certain biological parameters of *Trichogramma cacoeciae* Marchal 1927 (Hymenoptera: Trichogrammatidae) on *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) eggs and found similar results with the results of the current study. He reported that in the temperatures 20 °C, 25 °C and 30 °C, the mean numbers of eggs laid on the first day were, 4.2, 8 and 11; second day were 3, 7.7 and 7; and on the third day were 3, 6.2, and 5, respectively. In addition, the findings of the current study is akin to that of Shirazi (2006) who recorded a systematic decrease in the numbers of eggs laid per female of *T. chilonis* on eggs of *C. cephalonica* over time in three constant temperatures (20 °C, 25 °C and 30 °C). the numbers of eggs laid on the first day were 30, 49, and 44 in 20 °C, 25 °C and 30 °C, respectively. On the second day, the numbers were reduced to 4, 20, and 20 in 20 °C, 25 °C and 30 °C.

4.6.3. Duration of oviposition, pre-adult, and adults stages of *Trichogramma evanescens* on eggs of *Ephestia kuehniella* at three constant temperatures

Temperature affected many growth stages of *T. evanescens* as shown in Table 4.15 and Figure 4.15. The mean oviposition period (days) ranged from 1.6 days in 30 °C to 2.13 days in 20 °C (Table 4.15). The mean oviposition period from 25 °C was 1.8 days. The mean ovipositon day was significantly influenced by temperature ($F = 5.914$, $df = 2, 42$, $P = 0.005$). Also, the mean egg-adult period (days) was significantly affected by constant temperatures ($F = 900$, $df = 2, 42$, $P <$

0.0001). The highest mean egg-adult period was 15.47 days from 20 °C, followed by 11.47 days and 7.46 days from 25 °C and 30 °C, respectively (Table 4.15).

The egg-male adult period (days) was significantly affected by different constant temperatures (F = 1103.375, df = 2, 42, $P < 0.0001$). The egg-male adult period ranged from 7.0 days in 30 °C, 11.4 days in °C to 15.50 days in 20 °C. Also, the egg-female adult periods (days) was also significantly influenced by diffent constant temperatures (F = 911.181, df = 2, 42, $P < 0.0001$). The highest egg-female adult period was 15.53 days from 20 °C, followed by 11.40 days and 7.53 days from 25 °C and 30 °C, respectively (Table 4.15).

The longevity (days) of adult males of *T. evanescens* was not significantly different (F = 1.966, df = 2, 42, $P = 0.153$). The longevity adult males ranged from 2.13 days in 20 °C to 1.80 days in 25 °C (Figure 4.15A). In addition, the longevity (days) of adult females of *T. evanescens* was not significantly different (F = 0.978, df = 2, 24, $P = 0.384$). The highest adult female longevity was 2.53 days from 25 °C followed by 2.47 days and 2.27 days from 20 °C and 30 °C, respectively (Figure 4.15B).

Table 4.15. Mean oviposition and from egg to adult period (days) of *Trichogramma evanescens* on eggs of *Ephestia kuehniella* at three constant temperatures

Temperature (± 1 °C)	**n**	**Oviposition period (± s.e)**	**Mean egg-male period (± s.e)**	**Mean egg-female period (± s.e)**
20	15	2.13 ± 0.091a	15.40 ± 0.13a	15.53 ± 0.14a
25	15	1.80 ± 0.107b	11.40 ± 0.13b	11.40 ± 0.13b
30	15	1.60 ± 0.131b	7.20 ± 0.11c	7.53 ± 0.13c

Means in the same column with the same letter(s) are not significantly different (Tukey HSD, $P < 0.05$). n – number of replications; s.e – standard errors of the means.

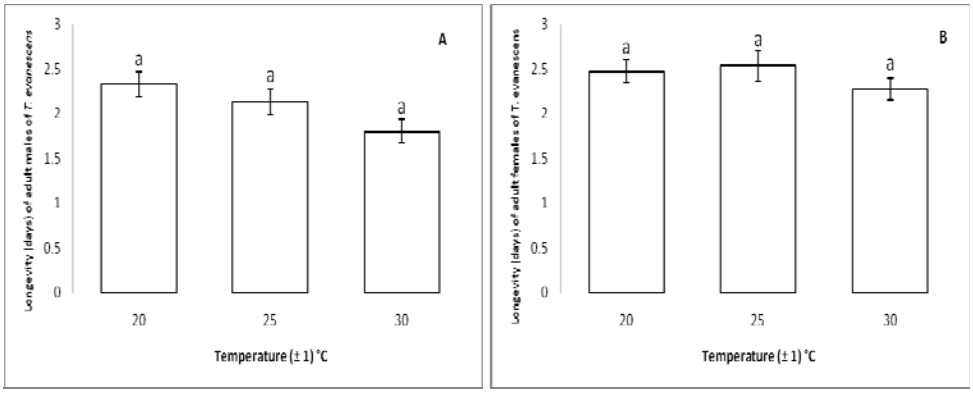


Figure 4.15. Longevity (days) of adult males and females of *Trichrogramma evanescens* on eggs of *Ephestia kuehniella* three constant temperatures. A – longevity (days) of adult males; B – longevity (days) of adult females. Mean numbers on the bars with the same letter(s) are not significantly different (Tukey HSD, $P < 0.05$).

Oviposition was successful in all three constant temperatures tested in this study. However, the duration varied considerably. The smallest oviposition time was observed from 30 °C, and the highest oviposition time from 20 °C.

Trichogramma evanescens was able to successfully complete its development from egg to adults on its prey *E. kuehniella* under all temperatures tested. The mean development time from egg-adult was significantly influenced by temperature. The mean egg-adult time increased with temperature, that is, temperature and mean egg-adult time showed an inverse relationship. The fastest egg-adult development time was from 30 °C. In fact, the mean egg-adult time of *T. evanescens* from 20 °C was twice that of 30 °C. This pattern was the same for mean egg-male and mean egg-female development. The result of this study are in accordance with those of Mohammad et al. (2015) who investigated some biological parameters of *T. evanescens* on eggs of lesser date moth under the constant temperature. Mohammade et al. (2015) reported that temperature and development time of *T. evanescens* had an inverse relationship. The mean egg-adult development time of *T. evanescens* on eggs of lesser date moth was 35, 20,

17, 10, 08, 07 and 00 days for temperatures of 15, 18, 20, 25, 28, 33 and 35 °C, respectively (Mohammad et al., 2015). Mawela et al. (2013) investigated the performance of *Trichogrammatoidae lutea* Girault (Hymenoptera: Trichogrammatidae) on three lepidopteran host and came to the same conclusion as in this study. Maceda et al. (2003) reported the same pattern for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and *Trichogramma annulata* De Santis (Hymenoptera: Trichogrammatidae), reared on eggs of *E. kuehniella* at constant temperatures of 15, 20, 25, and 30 °C. In the current sudy, the egg-male period was shorter than that of egg-female period although not significantly different for all the temperatures investigated. This hatching dynamic is very important for this species as it inhibits inbreeding and genetic decay by allowing males adequate time to move away and mate with females from different egg masses (Chuche and Thiery, 2012).

Even though adult longevity did not show statistical differences for males and females, it could be observed that longevity decreased with increased temperatures.

4.6.4. Proportion of female and male adult of *Trichogramma evanescens* reared on eggs of *Ephestia kuehniella* at three constant temperature

The proportion of female and male adult parasitoids that hatched from the eggs of *E. kuehniella* was influenced by the three constant temperatures: females ($F = 3.161$, $df = 2, 42$, $P = 0.053$) and males ($F = 119.411$, $df = 2, 42$, $P = 0.053$) (Figure 4.16). The proportion of females from 25 °C was highest (73.21%), followed by 20 °C (69.67%) and 30 °C (67.64%) (Figure 4.16A). The proportion of males from 30 °C (32.36%) was the highest, followed by 20 °C (30.34%) and 25 °C (26.79%) (Figure 4.16B).

Furthermore, t-test analyses revealed that the female and male proportions within a temperature was significantly different (Figure 4.16C). For 30 °C, female proportion was significantly higher than that of males ($t = 19.60$, $df = 28$, $P <$

0.0001). The proportion of female was 69.67% and that of male was 30.33% at 30 °C. The pattern was same for 25 °C (t = 15.93, df = 28, $P < 0.0001$), where the proportion of female was 73.21% and that of male was 26.79%, and 20 °C (t = 21.39, df = 28, $P < 0.0001$), where the proportion of female was 67.63% and that of male was 32.37%.

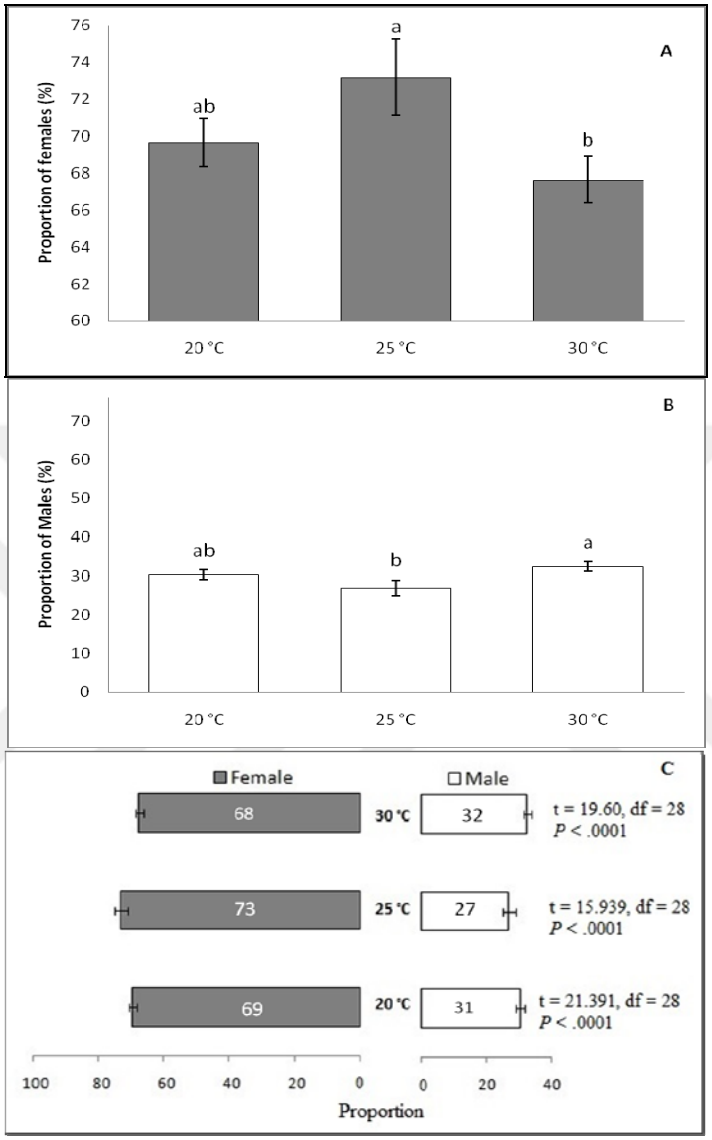


Figure 4.16. Proportions (%) of female and male adult of *Trichogramma evanescens* reared on eggs of *Ephestia kuehniella* in three constant temperature. A – proportions of females from three constant temperatures; B – proportions of males from three constant temperatures; C – proportions of female and male within a constant temperature.

This study reveals that among other life history traits of *T. evanescens*, temperature slightly influenced the sex ratio. The female and male proportion across temperatures was not strongly different (P = 0.053 – this could also be considered nonsignificant). The results in this study are similar to those of Krechemer and Foerster (2015), who investigated the effect of four constant temperatures (15, 20, 25 and 30 °C) on 3 *Trichogramma* species; *Trichogramma atopovirilia* Oatman and Platner (Hymenoptera: Trchogrammatidae), *Trichogramma acacioi* Brun (Hymenoptera: Trichogrammatidae) and *T. pretiosum* on eggs of the cabbage pest *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). They found no significant differences in the sex ratio across temperatures for each *Trichogramma* sp. Mawela et al. (2013) did not also find any significant differences in the sex ratio of *T. lutea* across temperature from 21 – 30 °C on eggs of three lepidopteran pests; *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), *C. partellus*, and *Cadra cautella* Walker (Lepidoptera: Pyralidae).

In this study, it can be observed that the proportion of females was higher than that of males. Such was the case in Krechemer and Foerster (2015), with the female:male ratios greater than 0.60. Mawela et al. (2013) also recorded female:male ratios greater than 0.60 on host of *C. partellus* and *C. cautella*, but recorded a female:male ratio of about 0.50 for the host *H. armigera* for all temperetures tested. Maceda et al. (2003) observed a female:male ratio for *T. pretiosum* on eggs of *Anagasta kuehniella* [*E. kuehniella*] of 0.73, 0.82, 0.91 and 0.52 for temperatures of 15, 20, 25, and 30 °C, respectively. High sex (female:male) ratios (> 0.60) seem to a widespread in many *Trichogramma* wasps. Muli et al. (2010) recorded high sex (female:male) ratios of 0.90, 0.91, 0.84, 0.77, 0.76, and 0.79 for *Trichogrammatoidea* sp. nr *lutea* reared on some lepidopteran pest namely; *B. fusca*, *S. calamistis*, *Mussidia fiorii* Cecconi and de Joannis (Lepidoptera: Pyralidae), *Mussidia* 'madagascariensis' (Lepidoptera: Pyralidae), *E. saccharina* and *C. partellus*, respectively in conditions of 26 ± 1 °C, and 50-70% RH.

4.7. Susceptibility of eggs and first instar larvae of *Chilo partellus* to some selected insecticides

4.7.1. Effects of insecticides on hatching of 'blackhead' egg masses

The result shows the effect of various insecticides on hatching of 'blackhead' egg masses of *C. partellus* (Table 4.16). The results revealed that there was a significant difference ($F = 41.23$, $df = 8, 18$, $P < 0.0001$) in the mean percentage of hatched egg mass from the different insecticides. The smallest percentage larvae (30.62%) was observed from deltamethrin application. This value was followed by the percentage hatching of chlorpyrifos-ethyl (86.32%) and lambda-cyhalothrin (89.93%). The hatching percentages for the other insecticides were also all greater than 90.0%. The numbers of emerged larvae for spinosad were 88, deltamethrin (33), lambda-cyhalothrin (88), emamectin-benzoate (94), indoxacarb (88), chlorpyrifos-ethyl (86), chlorantraniliprole (102), novaluron (105), and control (118).

Table 4.16. Mean percentage hatched 'black head' eggs of *Chilo partellus* after 24 hrs of application of insecticides

Treatment	n	eggs/mass	Mean ± sem	95% CI	
				Lower	Upper
Control	3	35–40	98.21 ± 0.65c	97.26	99.17
Spinosad	3	30-34	91.56 ± 2.07bc	88.17	94.98
Deltamethrin	3	32–40	30.62 ± 5.76a	21.67	39.58
Lambda-cyhalothrin	3	30–40	89.93 ± 1.20bc	87.99	91.88
Emamectin-benzoate	3	30–36	95.81 ± 0.88bc	94.51	97.12
Indoxacarb	3	30–32	98.96 ± 0.50c	97.91	98.96
Chlorpyrifos-ethyl	3	30–37	86.32 ± 1.93b	83.35	89.29
Chlorantraniliprole	3	32-40	95.06 ± 1.85bc	92.20	97.92
Novaluron	3	30–40	97.78 ± 1.02c	95.55	97.78

Eggs/mass refers to the number of eggs per egg mass on which topical application of insecticide was done. sem – Standard error of the means. 95% confidence interval (CI). Means in the same column with the same letter(s) are not statistically significantly different by Tukey HSD at $p < 0.05$.

It is known that the chorion layer of lepidopteran egg is not very permeable to ovicidal and toxic chemicals; nevertheless, some chemicals can pass through. In such events, these toxic chemicals can negatively affect embryonic development or result in death (Trisyono et al., 2000; Galvan et al., 2005). Of all the insecticides, deltamethrin significantly prevented hatching of the 'blackhead' egg masses (Table 2). On average, hatching was approximately three times lower in deltamethrin relative to the other insecticides. It is not clear why there is discrepancy in the percentage hatching between deltamethrin and lambda-cyhalothrin as they are members of the same chemical class.

Pineda et al. (2004) reported that spinosad diluted in water did not exhibit any ovicidal activity against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) eggs; however, when diluted in acetone, the percentage hatching was significantly reduced. In another related research conducted by Boiteau and Noronha (2007), the percentage of 'blackhead' egg masses of *O. nubilalis* hatching sprayed with indoxacarb, novaluron, spinosad, and water (as control) were not significantly different. On the other hand, when the insecticides were sprayed 2 days prior to the 'blackhead' stage, the percentage hatchability was very significantly reduced than when applied directly on the 'blackhead' egg masses by 60.0%, 80.0% and 80.0% for indoxacarb, novaluron, and spinosad, respectively. Mahmoudvan et al. (2014) also reported that indoxacarb SC (300 mgL^{-1}) and spinosad (480 mgL^{-1}) caused ovicidal (egg mortality) percentages of 86.02% and 100.0%, respectively on the egg masses of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Hexaflumuron (200 mgL^{-1}), and lufenuron (1000 mgL^{-1}) which are in the same group as nuvaluron, also caused ovicidal percentages of 100.0% and 49.55%, respectively in the same experiment. It is should be stressed that in Mahmoudvan et al. (2014) experiments, the egg masses used were less than or equal to 10hrs old. High ovicidal effect of novaluron against other lepidopteran has been reported (Assal et al., 1983; Chockalingan and Noorjahan, 1984).

At the commercial recommendations, the percentage hatching of lepidopteran egg mass is severely influenced by the solvent (medium) of the insecticide and the age of egg mass. De Smedt et al. (2015) indicated that hydrophobic compounds are more likely to absorb the intermediate and nonpolar poly (*p*-phenylene) PPPs, and therefore can easily attach to the hydrophobic egg surface, causing desiccation of the eggs. For this reason perhaps an increased hatching percentage could have been observed had a hydrophobic solvent like acetone and young egg masses (hours old) had been used.

4.7.2. Mortality of hatched larvae of *Chilo partellus* 48hrs after hatching

Chi-square analysis was used to compare the proportions of dead and alive larvae 48hrs after hatching (Figure 4.17). The proportions were significantly different ($P < 0.05$) for all insecticides except spinosad and chlorantraniliprole. The proportion of dead larvae was significantly higher in chlorpyrifos-ethyl (84.44%), emamectin-benzoate (87.48%), and deltamethrin (72.78%). Nevertheless, the proportion of dead larvae was significantly lower in novaluron (30.47%), indoxacarb (23.21%), and lambda-cyhalothrin (38.20%). Even though the proportion of dead and alive larvae was not significantly different in chlorantraniliprole and spinosad, it is worth mentioning that the proportions of dead larvae were higher than the proportion of alive larvae in both insecticides. The mortality rate of larvae 48hrs post hatching was also compared across the different insecticides (Figure 4.18). The percentage mortality was significantly different ($F = 34.30$, $df = 8, 18$, $P < .0001$) across the various insecticides. The highest percentage mortality was observed from emamectin-benzoate (84.48%), and followed by chlorpyrifos-ethyl (84.44%). The lowest mortality was recorded from indoxacarb (23.21%), followed by novaluron (30.47%) and lambda-cyhalothrin (38.20%).

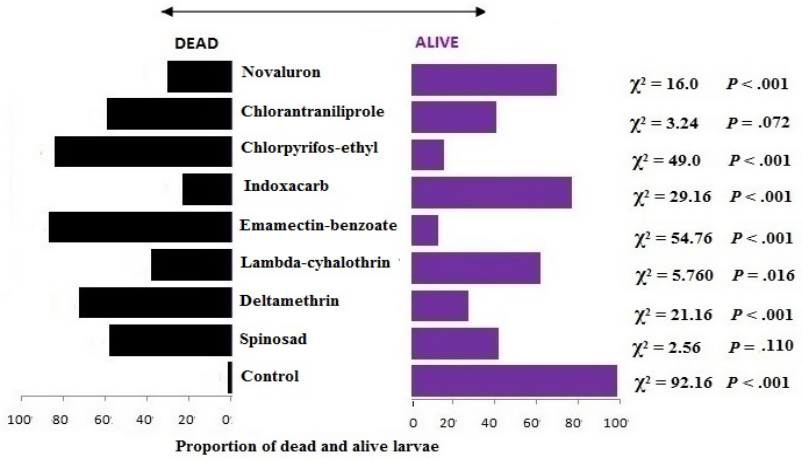


Figure 4.17. Mean percentage mortality of larvae *Chilo partellus* that hatched from insecticide treated eggs after 48 hours: comparing proportions alive and dead across the same treatment. Arrow shows direction of mean comparison.

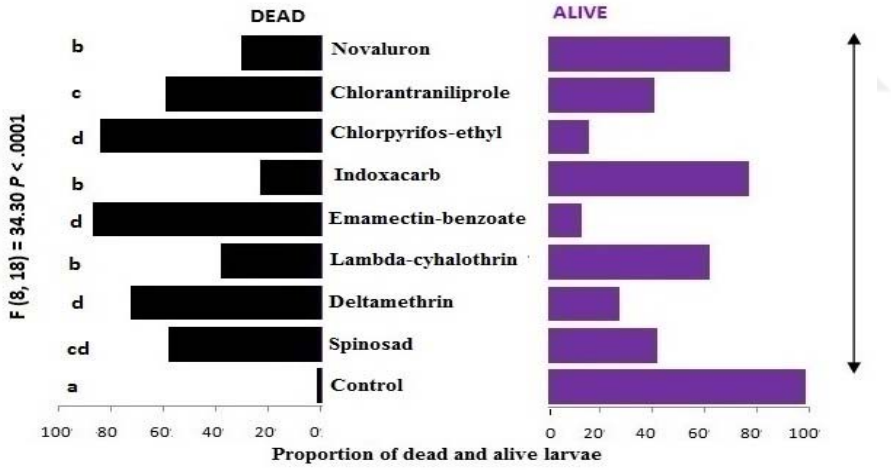


Figure 4.18. Mean percentage mortality of larvae of *Chilo partellus* that hatched from insecticide treated eggs after 48 hours: comparing percentages of dead and alive across different treatments. Bars with the same letter(s) are not statistically different (Tukey HSD, $\alpha = 0.05$). Arrow shows direction of mean comparison.

The survival of larvae hatched from the insecticide-treated egg masses was impacted by all insecticides. Emamectin-benzoate, chlorpyrifos-ethyl, deltamethrin, spinosad and chlorantraniliprole had higher larvicidal effects. The dead larvae were probably contaminated by these insecticides as they chew their way out of the egg masses through their mouth or by contact with contaminated surfaces. Indoxacarb is reported to have ovilarvicidal properties against some pests such as codling moth and *O. nubilalis* (Boiteau and Noronha, 2007), however, this was not the case in the current study.

4.7.3. Effects of insecticides on first instar larvae of *Chilo partellus*

The effect of the various insecticides on the first instar larvae 72hrs post application is given in Figure 4.19. The effect was measured as the number of larvae dead (percentage mortality). The insecticides significantly influenced ($F = 8.256$, $df = 8, 18$, $P < 0.0001$)

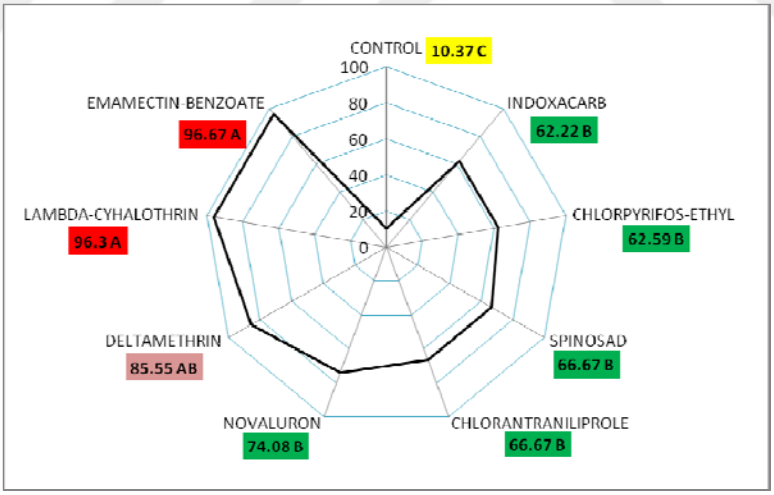


Figure 4.19. Mean percentage mortality of first instar larvae of *Chilo partellus* 72 hours post exposure to insecticide-infested leaf discs (*percentage mortality was transformed using arcsine transformation before analysis)*. Means in the radar chart with the same letter(s) and colours are not statistically different (Tukey HSD, $\alpha = 0.05$).

The effect of insecticides on the first instar larvae in this study revealed that the first instar larvae were very susceptible to the insecticides used in this study. Emamectin-benzoate, pyrethroids and spinosad are known to cause high larval mortality of lepidopteran pests such as *O. nubilalis, S. nonagrioides, Spodoptera exigua, P. xylostella* (Pineda et al., 2004; Boiteau & Noronha 2007; Kurt and Kayis, 2014; Mahmoudvan et al., 2014) including *C. partellus* (Tanwar et al., 2017; Kumar and Alam, 2017). Intoxication of the larvae possibly came from contamination with the leaves as they wander around, and through feeding.

4.7.4. Feeding behavior of first instar larvae of *Chilo partellus* on insecticide treated leaf discs after 72 hours

At the end of the study (72hrs), the number of patches on the insecticide treated leaves due to larval feeding were counted and scored. No feeding was observed on leaves treated with deltamethrin, lambda-cyhalothrin, emamectin-benzoate and chlorantraniliprole after 72 hours. The scored values were subjected to Kruskal-Wallis ANOVA. The mean scores; indicated by the mean rank was significantly different ($\chi 2 = 18.793$, df = 8, $P < 0.016$). The highest mean rank was obtained from the control and novaluron treatments. The smallest mean ranks were recorded from deltamethrin, lambda-cyhalothrin, emamectin-benzoate, and chlorantraniliprole (Table 4.17). The highest reduction in feeding damage by first instar larvae of *C. partellus* compared to the control (%) was obtained from leaves treated with deltamethrin, lambda-cyhalothrin, emamectin-benzoate, and chlorantraniliprole.

Table 4.17. Mean number of patches fed on and reduction in feeding damage by first instar larvae of *Chilo partellus* on insecticide treated leaves after 72 hours

Treatment	n	True mean (± sem)	Mean ranks	Reduction in feeding damage relative to control (%)
Deltamethrin	3	0.0 ± 0.0	9 a	100
Lambda-cyhalothrin	3	0.0 ± 0.0	9 a	100
Emamectin-benzoate	3	0.0 ± 0.0	9 a	100
Chlorantraniliprole	3	0.0 ± 0.0	9 a	100
Spinosad	3	0.33 ± 0.3	13.17 b	85.84
Indoxacarb	3	0.33 ± 0.3	13.17 b	85.84
Chlorpyrifos-ethyl	3	0.67 ± 0.3	17.33 bc	71.24
Novaluron	3	1.02 ± 0.5	21.50 bc	57.08
Control	3	2.33 ± 0.7	24.83 c	0

sem – standard error of the means. Mean ranks with the same letter(s) are not statistically significantly different (Kruskal Wallis test, Dunn's post hoc mean separation test, $\alpha = 0.05$).

Results from the feeding behaviour experiment revealed that these insecticides have the potential to reduce the feeding activity of first instar larvae, thereby ensuring adequate photosynthesis for the plants. In this study, deltamethrin, lambda-cyhalothrin, emamectin-benzoate exerted the greatest negative effect on the feeding capability of the first instar larvae. In a related study, Hannig et al. (2009) investigated the effect of chlorantraniliprole, methomyl, esfenvalerate, lambda-cyhalothrin, indoxacarb, emamectin-benzoate, methoxyfenozide and metaflumizone on the feeding behaviour of four lepidopteran species: *P. xylostella*, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). They recoreded foliar damage (mm^2) of 0.1, 0.1, 0.3, 0.7, 3.3, 5.5 and 71.9 mm^2 by larvae of *S. exigua* on leaves treated with methynol, esfenvalerate, lambda-cyhalothrin, Chlorantraniliprole, indoxacarb, emamectin-benzoate and a control (distil water), respectively, after 48 hours. They also reported the reduction in

feeding damage relative to the control treatment (%) as 99.8, 99.9, 99.5, 99.0, and 75.4 % by larvae of *S. exigua* on leaves treated with methynol, esfenvalerate, lambda-cyhalothrin, Chlorantraniliprole, indoxacarb and emamectin-benzoate, respectively, after 48 hours. They pattern was the same for *H. zea*, *P. xylostella*, and *T. ni*. With regards to time to feeding cessation and reduction in feeding, chlorantraniliprole was the fastest-acting insecticide, ahead of emamectin-benzoate, indoxacarb, lambda-cyhalothrin, esfenvalerate, and methomyl. The findings of Hannig et al. (2009) are in concordance with the current study.

4.7.5. Survival analysis

The mortality of the first instar larvae was monitored every 12 hour. Figure 4.20 shows the Kaplan-Meier curve and Table 4.18 shows the median time (LT_{50}) and the hazard ratios (HR) with their corresponding 95% confidence interval (CI) for each insecticide.

The steepness of the slope started in the 48th hour for deltamethrin, with 4 larvae surviving to the end of the study. The steepness for lambda-cyhalothrin is similar to that of deltamethrin; however, only 2 larvae survived to the end of the study. The steepness began in the 24th hour for emamectin-benzoate with 1 larvae surviving to the end of the study. The steepness of the slope for indoxacarb started in the 12 hour with 11 larvae surviving to the end. The steepness of the slopes for chlorpyrifos-ethyl and chlorantraniliprole started in the 36th hour with 11 and 10 larvae surviving to the end, respectively. Novaluron also had a steep slope beginning from the 36th hour with 7 larvae surviving to the end (Figure 4.20).

The reference treatment in this study was the control. Since the mortality in the reference treatment was never up to 50.0% in any replication in the course of the study, it was practically impossible to obtain median time values for the control. The median time ranged from 36-72 hours. The shortest median time was recorded from emamectin-benzoate (36.0 hours, 95% CI: 27.33 – 44.68). The highest median time was recorded from indoxacarb (72 hours, 95% CI: 56.67-

87.33) and chlorantraniliprole (72 hours, 95% CI: 61.88-82.121). The median time for all other insecticide was 60 hours. For spinosad, the mortality slope was gentle, with 11 larvae surviving to the end of the study. The hazard ratios in this study ranged from 4.91 (95% CI: 1.66 – 14.58, $P < 0.0001$) from chlorpyrifos –ethyl to 15.584 (95% CI: 5.331 – 45.557), $P < 0.0001$) in emamectin-benzoate (Table 18). Survival analysis is widely used in scientific research to evaluate the rate of a certain outcome (mortality) over time. Our study revealed that emamectin-benzoate had the lowest median time and the highest hazard ratio.

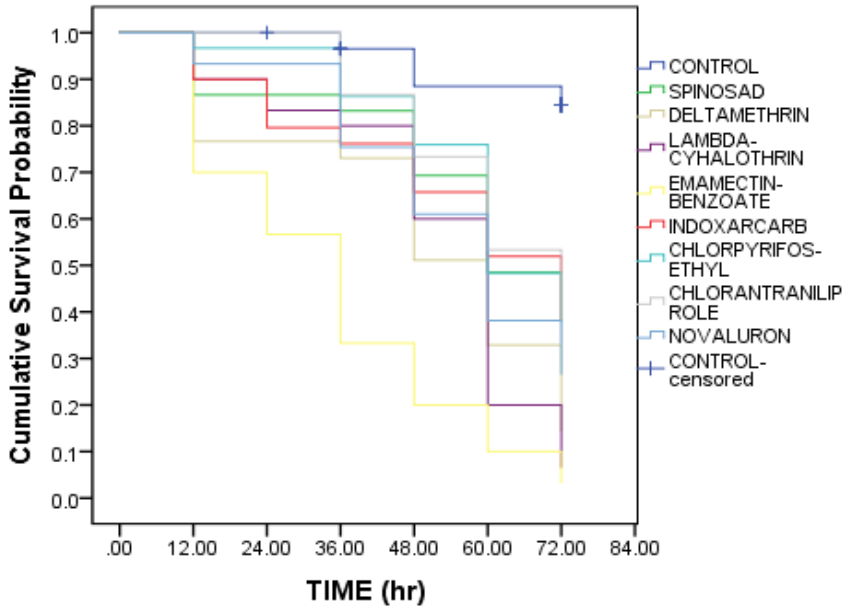


Figure 4.20. Kaplan-Meier survival curves for first instar larvae of *Chilo partellus* exposed to different insecticides treated leaf disks after 72 hours.

Table 4.18. Hazard Ratios (HR) and Median Time (LT50) (hours) for insecticides

	Hazard ratios and 95% CI		Median time and 95% CI		*P*
Treatment	**HR**	**95% CI**	**LT_{50} (hours)**	**95% CI**	
Control	-	-	-	-	-
Spinosad	5.13	1.73 – 15.18	60.0	47.15 – 72.8	.003
Deltamethrin	8.82	3.04 – 25.62	60.0	49.56 – 70.44	.0001
Lambda-cyhalothrin	10.17	3.50 – 29.52	60.0	55.05 – 64.95	.0001
Emamectin-benzoate	15.58	5.33 – 45.56	36.0	27.33 – 44.68	.0001
Indoxacarb	5.36	1.82 – 15.93	72.0	56.67 – 87.33	.002
Chlorpyrifos-ethyl	4.91	1.66 – 14.58	60.0	50.09 -69.91	.004
Chlorantraniliprole	5.39	1.82 – 15.80	60.0	50.38 – 69.62	.002
Novaluron	6.70	2.28 – 19.72	60.0	56.32 – 63.68	.001

Omnibus Test for the Hazard Ratios [chi square $\chi 2 = 65.589$, df = 8, $p < .0001$], Log Rank for LT50 [chi square $\chi 2 = 80.617$, df = 8, $p < .0001$. CI – confidence interval.

4.7.6. Cluster analysis

A hierarchical cluster analysis was conducted so as to group the insecticides based on the parameters studies namely; percentage hatchability of 'blackhead' egg masses, mortality of hatched larvae from insecticide-treated 'blackhead egg masses, mortality of first instar larvae fed with insecticide-treated leaves, median times (LT50) for first instar larvae fed with insecticide-treated leaves, the hazard ratios of insecticides against first instar larvae fed insecticide treated leaves and the feeding behaviour of first instar larvae fed insecticide-treated leaves. Since the data from the different parameters were on different scales, the values were standardized from -1 to +1. The data was classified using the centroid linkage distance. The cladogram revealed 4 distinct groupings of the insecticide (Figure 4.21). Emamectin-benzoate, lambda-cyhalothrin, and deltamethrin formed single groups each. The other insecticides were very similar, hence formed one single group.

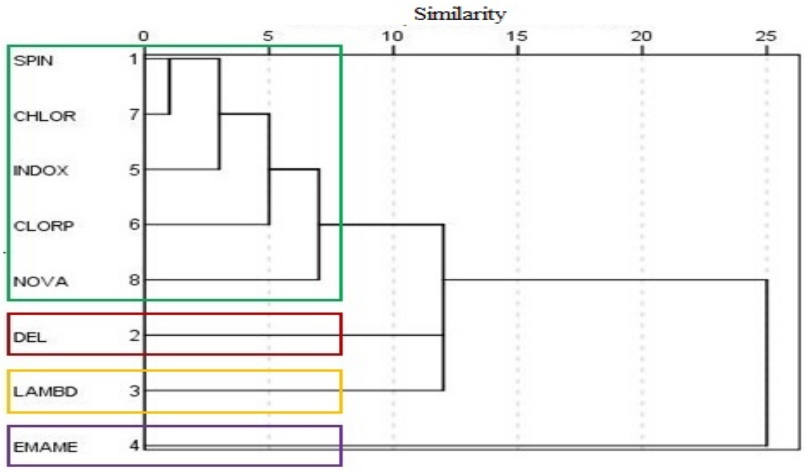


Figure 4.21. Insecticide clustering based on six variables: Hatchability, Mort48, Mort72, Median, HR and Feeding behaviour. The number of clusters was selected based on the elbow method. Number of cluster was based on the elbow-rule. Key: SPN – Spinosad, DEL – Deltamethrin, LAMBD – Lambda-cyhalothrin, EMAME – Emamectin-benzoate, INDOX – Indoxacarb, CLORP – Chlorpyrifos-ethyl, CHLOR – Chlorantraniliprole, NOVA – Novaluron.

In an attempt to group these insecticides based on the totality of their performance on the parameters examined in this study, emamectin-benzoate, followed by lambda-cyhalothrin and deltamethrin stood out singly. The other group consisted of chlorantraniliprole, spinosad, chlorpyrifos-ethyl, indoxacarb and novaluron. Chlorantraniliprole, spinosad, indoxacarb and novaluron are in the group of insecticides described as reduced-risk insecticides (Hannig, 2009; Boiteau and Noronha, 2007). They showed higher mortality percentages in comparison to traditional insecticides such as chlorpyrifos-ethyl. In 2012, Rameash et al. (2012) from India reported that two foliar application of novaluron, spinosad, and emamectin-benzoate reduced *C. partellus* infestation of field maize to 15.0%, 6.67%, and 8.33% in the first crop and 10.0%, 5.0%, and 6.67% in the second crop, respectively.

4.8. Molecular characterization of *Chilo partellus* populations in Turkey

4.8.1. Gel electrophoregram of cytochrome oxidase subunit I (COI) of mtDNA

The electrophoregram of PCR based amplication products of *C. partellus* populations collected from four provinces in Turkey and one province (Cape Town) in South Africa is shown in Figure 4.22. The DNA extraction-PCR-Gel electrophoresis processes were done in two shifts (Figure 4.22. a and b) for logistics reasons and optimization of the processes. Both shifts resulted into clear observable bands which were estimated at ~ 710 bp.

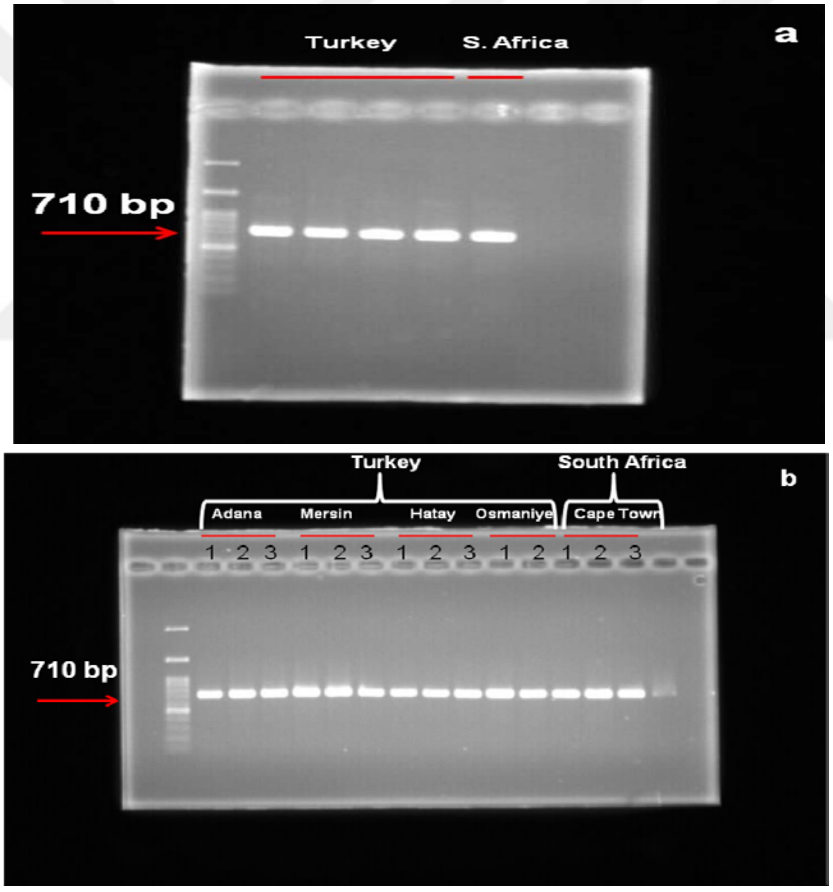


Figure 4.22. Electrophoregram of *Chilo partellus* populations collected from four provinces in Turkey and one province in South Africa by using the LCO1490 and HCO2198 primers. a – first shift; b – second shift.

4.8.2. Species confirmation and phylogenetic reconstruction

The mtDNA COI gene region of the specimens of *C. partellus* collected in South Africa (Cape Town) and Turkey were analysed by comparing them with already confirmed *C. partellus* mtDNA COI gene sequence in the GenBank of NCBI database from India, Pakistan and Kenya. The sequence identification search confirmed that all samples used in this study significantly matched the identity of published *C. partellus* sequences with a 99-100% nucleotide identity.

The phylogenetic tree of *C. partellus* populations in Turkey and South Africa, with publicly available authenticated barcodes of *C. partellus* in the GenBank of NCBI database, with *O. nubilalis* as an outgroup is given in Figure 4.23. The sequences used were trimmed from 710 bp of the specimens and the phylogenetic tree constructed using ML. The ML grouped the *C. partellus* sequences in the current study into three clades after a 1000 bootstrap replication. The first clade is consisted of KX351380.1 (Meghalaya, India), Osminiye-2, Hatay-2, Hatay-1, Adana-2 and Mersin-1 (Turkey). The second clade is consisted of MK566473.1 (Kenya), Adana-1, Mersin-2, Osmaniye-1 (Turkey), and South Africa-1, South Africa-2 (South Africa). The third clade is composed of HQ991339.1 (Manakpur, India), HQ991339.1, KP233794.1 (Pakistan), HQ991286.1, HQ991263.1, HQ991218.1, HQ990908.1 HQ990905.1 (Pakistan).

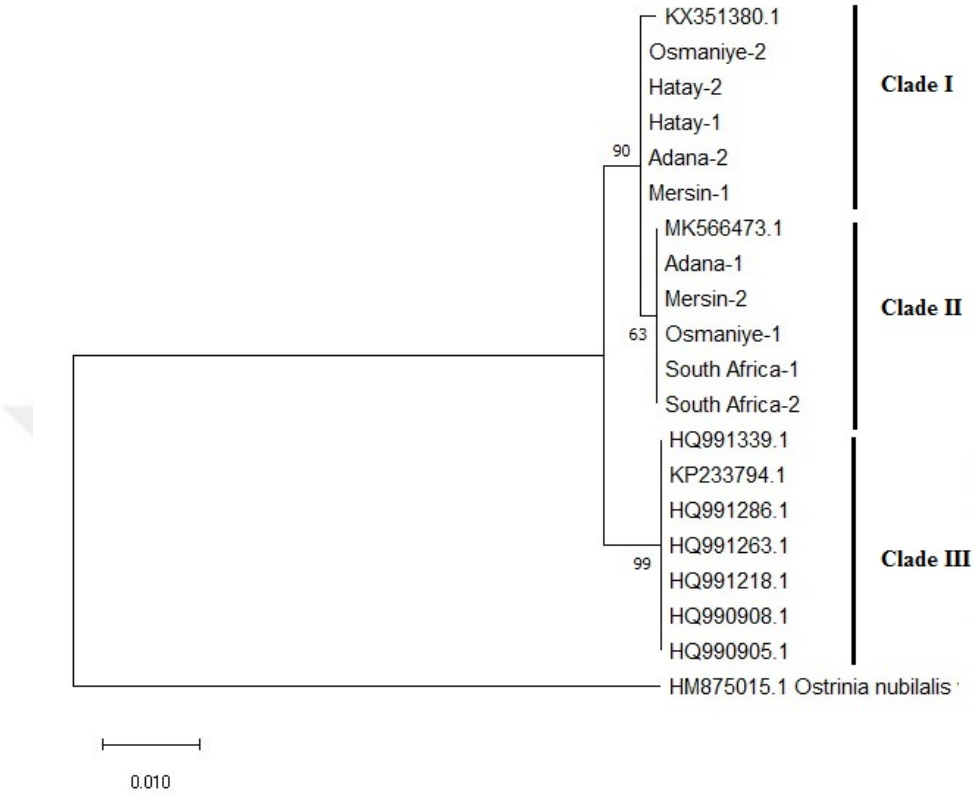


Figure 4.23. Phylogenetic tree constructed using Maximum Likelihood (ML) method of mtDNA CO1 region of *Chilo partellus* and *Ostrinia nubilalis* sequences obtained from maize fields in Turkey and South Africa together with references from India, Pakistan and Kenya. The reference sequences were obtained from the GenBank of NCBI database. The tree is based on the Kimura 2-parameter method with 1000 bootstrap replicates. Bootstrap support values are given on the branches.

4.8.3. Origins of Turkish population of *Chilo partellus*

According to the phylogenetic tree (Figure 4.23) constructed in this study, the route of invasion of the *C. partellus* to Africa can be hypothesized as shown in Figure 4.24. This suggest that the Turkish population of *C. partellus* is a mixed population originating from Southern - Eastern Africa (South Africa and Kenya) and from the Indian subcontinent (India).

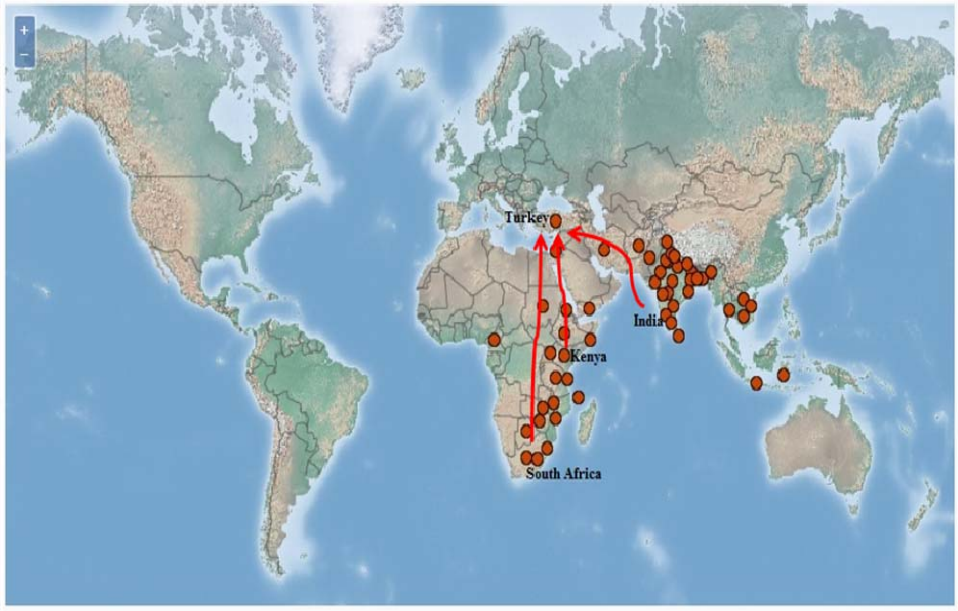


Figure 4.24. World map showing possible invasion route of Turkish population of *Chilo partellus* to Turkey

According to the electrophoregram of the COI mtDNA gene observed in this study, the fragments were ~ 710 bp for all samples. Given the primers used, 710 bp is within the range of many lepidopteran pests of maize. Assefa et al. (2015) used the thoraces of adults of *B. fusca* under the same conditions and recorded a band size of 525 bp. A 710 bp fragment was recorded for *B. fusca* by Peterson et al. (2016). An average of 583 bp for *C. partellus*, *B. fusca*, and

Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) was recorded in Malawi (Donga and Meadow, 2018).

The cytochrome oxidase subunit I (COI) gene of mtDNA is widely used to identify and study population genetics, analysing phylogenetic relationships in insects (Sezonlin et al. 2006; Calatayud et al. 2011; Assefa et al. 2016), and particularly to examine intra and inter-species interactions in maize stem borers (Le Rue et al. 2014; Donga and Meadow, 2018; Goftishu et al. 2018). All specimens examined in this study were *C. partellus*, confirmed when compared to already published sequences in the GenBank of the NCBI database to about 99-100% identity accuracy. The specimens of Turkish populations of *C. partellus* and South African were grouped into two clades, each containing mixed populations from Turkey. Specimens from South Africa were all grouped in clade II alongside the reference sequence (MK566473.1) from Kenya. The reference sequence (KX351380.1) from India was grouped in clade I. The phylogenetic tree constructed indicates that Turkish population of *C. partellus* is a mixed population from India and southern and eastern Africa. In addition, it can also be inferred that Turkish population of *C. partellus* has not maintinaed strick geographical isolation as per the source population, rather, has spread throughout the Mediterranean belt of Turkey and interbreed. According to Lesieur et al. (2016) this interbreeding situation and sharing of genetic material of Turkish populations of *C. partellus* can reduce cost of control and management, since very genetically diverse populations of pests differ in their responses to several pest management strategies.

While a definitive route of invasion of *C. partellus* in Turkey cannot be described at this point, it can be hypothized (based on the comparison with already published accession on the GenBank of NCBI) that Turkish populations of *C. partellus* are from Africa and Asia. Long-distance migration of lepidopteran stem borers is common (Goftishu et al. 2018; Assefa, 2019), however, it is unclear if the Turkish population of *C. partellus* is as a result of long-distance migration or arrival of contaminated commodity.

5. CONCLUSION AND RECOMMENDATIONS

Within the scope of this thesis titled 'Some bionomic parameters, molecular characterization, and susceptibility of spotted stem borer *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae) to selected insecticides in Adana province, Turkey', the results and recommendations are presented below.

1. In 2018, the first observation of *C. partellus* larvae on the first crop of maize was in the first week of May. A major peak population of 6.1 larvae/plant of *C. partellus* was recorded on 10 May. The first observation of *O. nubilalis* and *S. nonagrioides* was on 28 June and 19 July. The mean population of *O. nubilalis* and *S. nonagrioides* remained low (below 2 larvae per plant) compared to *C. partellus* through the first crop of maize in 2018. The first observation of *C. partellus* larvae on the second crop of maize in 2018 was on 05 July. A peak of 5.75 *C. partellus* larvae/plant was observed on 5 July. A peak population of larvae of *O. nubilalis* and *S. nonagrioides* larvae/plant was observed on 16 August.
2. In 2019, the first *C. partellus* larva on maize plant was observed on 15 May. A major peak population of 0.45 larvae per plant was observed on 28 May on the first crop of maize. The first observation of *O. nubilalis* and *S. nonagrioides* larvae/plant on the first crop of maize was on 26 June and 10 July, respectively.
3. In the second crop of maize of 2019, a major peak population of 2.27 larvae of *C. partellus* per plant was recorded on 31 July. The population of *O. nubilalis* rose and peaked on mid September with a mean of 4.7 larvae. The population of *S. nonagrioides* remained relatively low for the entire second crop of maize in 2019.

4. In 2018, the first flight trap catch of *C. partellus* on the first crop of maize was on 26 April. For the second crop of maize in 2018, the first flight was observed on 05 July.
5. In 2019, the first flight of *C. partellus* on the first crop of maize was on 03 May. Two peaks were observed; one on the vegetative stage and the other on the dry stage of maize. For the second crop of maize in 2019, the first flight was on 24 July.
6. In 2018, the first sign of stem borer infestation on the first crop of maize was observed on 10 May. The percentage increased from 26.67% (10 May) to 40.0% (24 May). The highest infestation was in the mature stage of maize. In the second crop of maize in 2018, the percentage increased from 13.3% (5 July) to 96.61% (19 July). In 2019, the first observation of stem borer infestation on the first crop of maize was observed on 15 May at 3.3%. In the second crop of maize of 2019, the percentage rose from 10.16% (24 July) to 76.27% (31 July). After 07 August (66.67%), the percentage infestation remained high above 90.0% through the entire study.
7. Positive regression models were observed for the mean numbers of the three major stem borers and the percentage of maize plant infestation.
8. In the first crop of maize in 2018, leaf and stem damage by the major stem borers were 60.61% and 39.39%, respectively in the vegetative stage of maize. In the tassel+cob stage, the leaf, stem, tassel and cob damage by major stem borers were 42.93% and 39.63%, 4.44%, 12.96%, respectively. In the dryness stage, leaf, stems, tassel, cob damage by major stem borers were 44.30%, 29.55%, 23.74% and 2.41%.
9. In the second crop of maize in 2018, the damage on leaf and stem by major stem borers of maize were 62.35% and 37.64%, respectively, in the vegetative stage. In the tassel+cob stage, leaf, stem, tassel and cob damage by major stem borers were 37.70%, 37.81%, 3.44% and 21.05%,

respectively. In the dryness stage, the damage on leaves, stems, cobs and tassels were 33.17%, 39.77%, 22.11% and 4.95%.

10. In the first crop of maize in 2019, the percentage of damaged leaves and stems were 68.00% and 32.00%, respectively, in the vegetative stage of maize. In the tassel+cob stage, the damage on stems, leaves, cobs and tassel were 51.77%, 26.95%, 16.31% and 8.00%. In the dryness stage, the percentage of damaged stems, cobs, leaves and tassel were 57.41%, 24.07%, 9.26% and 9.36%, respectively.
11. In the second crop of maize in 2019, the percentage of damaged on leaves and stems were 76.21% and 23.24%, respectively, in the vegetative stage. In the tassel+cob stage, the percentage of damaged on stem, leaves, cobs and tassel were 38.92%, 34.18%, 23.18% and 8.0%, respectively. In the dryness stage in the second crop of 2019, the percentage of damage on stems, leaves, cobs and tassels were 57.41%, 16.88%, 26.69% and 3.18%, respectively.
12. The Taylor aggregation index (b) for the different stages of *C. partellus* at different maize phenology in the first crop of maize in 2018 were greater than 1. The Taylor aggregation index (b) for the second crop of maize for the larval+pupal stages were -0.181, 1.426 and 1.154 in the vegetative, maturity and dryness stages of maize, respectively.
13. The Taylor aggregation index (b) for the different stages of *C. partellus* at different maize phenology in the first crop of maize in 2019 were greater than 1.
14. The optimum sample units (N) for maize at different population densities of *C. partellus* in the vegetative stage of the first crop of maize of 2018 were, larvae+pupae density of 1/plant requires 1374, 344 and 153 maize sample units at precision levels of 0.10, 0.20, and 0.30, respectively. At the vegetative stage of the second crop of 2018, a larvae+pupae density of

1/plant required 774, 193, and 86 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively

15. The optimum sample units (N) for maize at different population densities of *C. partellus* in the vegetative stage of the first crop of maize of 2019 were, a larvae+pupae mean density of 1/plant requires 1045, 261 and 116 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively. At the vegetative stage of the second crop of maize of 2019, a larvae+pupae density of 1/plant requires 256, 64, and 28 maize sampling units at precision levels of 0.10, 0.20, and 0.30, respectively.
16. In 2018, the first parasitized egg masses were observed on 09 August with a parasitism rate of 71.43%. Parasitism rates reached 100% on 16 August and 11 September.
17. In 2019, the first parasitism of egg masses of *C. partellus* was observed on 31 July at a rate of 20.0%. The highest parasitism rate (50.0%) was observed on 18 September.
18. The parasitoid species of this study was identified as *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on the bases of morphological characters of male genitalia and male antennae.
19. The first peak occurred on 11 May and 21 May in 2017 and 2018, respectively, corresponding to the accumulation of 12 and 24 degree-days, respectively. The second peak in 2017 and 2018 started on the same week, (~ 5 July), corresponding to the accumulation of 116 degree-days. The second peak ended on 05 and 12 October in 2017 and 2018, representing an accumulation of 372 and 387 degree-days, respectively.
20. In the evaluation of the performance of *T. evanescens* on eggs of *Ephestia kuehniella*, highest mean number of eggs laid per female of *T. evanenscens* was 43.87 from 25 °C, followed by 41.80 and 28.13 from 20 °C and 30 °C, respectively.

21. The percentage number of larvae of *T. evanescens* that hatched from the prey eggs were 86.22%, 92.43% and 86.28% at 20 °C, 25 °C and 30 °C. The mean oviposition period (days) ranged from 1.6 days in 30 °C, 1.8 days in 25 °C to 2.13 days in 20 °C. The mean egg-adult period were 15.47 days from 20 °C, followed by 11.47 days and 7.46 days from 25 °C and 30 °C, respectively. The adult female longevity of *T. evanenscens* was 2.53 days from 25 °C followed by 2.47 days and 2.27 days from 20 °C and 30 °C, respectively.
22. The proportion of females of *T. evanenscens* from 25 °C was highest (73.21%), followed by 20 °C (69.67%) and 30 °C (67.64%). The proportion of males from 30 °C (32.36%) was the highest, followed by 20 °C (30.34%) and 25 °C (26.79%).
23. In the experiment to evaluate the effect of some commonly used insecticides on the blackhead stages of the eggs masses of *C. partellus*, the hatching percentages of blackhead eggs were 98.21%, 91.56%, 30.62%, 89.93%, 95.81%, 89.96%, 86.32%, 95.06% and 97.78% from control, spinosad, deltamethrin, lambda-cyhalothrin, emamectin-benzoate, indoxacarb, chlorpyrifos-ethyl, chlorantraniliprole and novaluron treatments, respectively.
24. The percentage mortality of first instar larvae of *C.partellus* after 72 hours of exposure to insecticide was 96.67%, 96.30%, 85.55%, 74.08%, 66.67%, 66.67%, 62.59%, 62.22% and 10.37% from emamectin-benzoate, lambda-cyhalothrin, deltamethrin, novaluron, chlorantraniliprole, spinosad, chlorpyrifos-ethyl, indoxacarb and control treatments.
25. Reduction in the feeding damage of the first instar larvae of *C. partellus* with 72 hours of exposure to some insecticides relative to the control treatment was 100% for deltamethrin, emamectin-benzoate, lambda-cyhalothrin and chlorantraniliprole.

26. From the survival analysis, the hazard ratios of the insecticides on the first instar larvae of *C. partellus* after 72 hours were 15.58, 10.17, 8.82, 6.70, 5.39, 5.13, 4.91, and 5.36 from emamectin-benzoate, lambda-cyhalothrin, deltamethrin, novaluron, chlorantraniliprole, spinosad, chlorpyrifos-ethyl and indoxacarb treatments, respectively.
27. In the experiment to characterize the Turkish populations of *C. partellus* using molecular techniques, all the bands in the gel electrophoregram of the cytochrome oxidase subunit I (COI) of the mt DNA were ~710 bp. The gel electrophoregram on the samples from South Africa (Cape Town) was 710 bp.
28. All specimens used in the current study were confirmed as *C. partellus* using mtDNA sequences from the GenBank of NCBI with a 99-100% nucleotide identity.
29. The phylogenetic tree of *C. partellus* populations in Turkey and South Africa constructed using maximum likelihood with publicly available authenticated barcodes of *C. partellus* in the GenBank of NCBI database, with *O. nubilalis* as an outgroup resulted into three distinct clades after 1000 bootstrap.
30. The ML phylogenetic tree suggests that suggest that the Turkish population of *C. partellus* is a mixed population originating from Southern - Eastern Africa (South Africa and Kenya) and from the Indian subcontinent (India).

This study reveals that *C. partellus* infestation of maize in Adana, Turkey starts earlier than other major stem borers of maize. This early infestation is a source of damage and yield loss. However, high parasitism of *C. partellus* by the native generalist parasitoid *T. evanescens* in field conditions can play a fundamental role in the management of *C. partellus*. From the findings of this study, the following are recommended:

- Parasitism of *T. evanenscens* on egg masses of *C. partellus* on maize crop grown commercially is not yet known. This issue needs further investigation.
- Evaluation of some biological parameters of *T. evanescens* on eggs of *C. partellus* is recommended.
- *Chilo partellus* showed high susceptibility to many commonly used insecticides under laboratory condition in Turkey. A better understanding of these insecticides requires a long-term (indirect and subtler effect) study on the physiology and behavior of the target pests and their natural enemies, thereby developing a formidable IPM strategy for *C. partellus* in the maize agroecosystem in Turkey.
- Additional experiments are therefore recommended to highlight and underscore the ecological effects of these insecticides in field trials. Points of focus could be on the dosage and time of application. Thus the role of novaluron and chlorantraniliprole should be considered alongside emamectin-benzoate, lambda-cyhalothrin and deltamethrin as part of an IPM plan. As a cultural practice, studies geared towards obtaining resistaint maize varieties to stem borer infestation in the region are recommended.
- Since the Turkish populations of *C. partellus* is hypothesized to have come from India, South Africa and Kenya, it is recommended that lessons be drawn from these host regions on the control and management of *C. partellus* and adapt to locally available techniques.

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RESUME

Tange Denis ACHIRI was born on 1988 in Ekonto Titi, Cameroon. He attended the Cameroon Baptist Convention (CBC) Primary in Ekondo Titi and eventually moved to Presbyterian High School (PHS) Kumba where he otained the General Certificate of Education (GCE) Ordinary Level in 2004 and Advanced Level in 2006. He earned his Bachelors Degree (BSc) in Zoology from the University of Buea Cameroon in 2010. In 2011 he was awarded the German Academic Exchange Service (Deutsche Akademische Austauschdienst (DAAD) scholarship which enabled him to study in the University of Ghana – Legon (Accra, Ghana), where he obtained a Master of Philosophy (MphiL) in Entomology from the African Regional Postgraduate Program in Insect Science (ARPPIS) in 2013.

Between 2014 – 2015, Tange Denis Achiri served as a Lecturer in the School of Agriculture and Veterinary Sciences in National Polytechnic Bamenda. During this period he also worked as a Freelance biostatistician to many students, lecturers and research institutions.

In 2015 he obtained a scholarship from the Government of Turkey (Türkiye Bursları) to pursue a PhD program in Plant Protection in the Department of Plant Protection, Çukurova University, Adana, Turkey.

His research area is focused on sustainable agriculture with interest in pest management and overall plant health. Achiri is an active researcher with a trail of publications in peer-reviewed journals. He is also a seasoned biostatistician and his passion for applied statistics (especially in agriculture) has led to a book publication in this area.