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DIVERSITY OF *TRICHOGRAMMA* WASPS ATTACKING *HELICOVERPA ARMIGERA* (HUBNER) IN MIXED VEGETATION ECOSYSTEMS OF ETHIOPIA

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ABSTRACT

The nature of interactions between insect pests, crops, non-host plants, and their physical environment determines the effectiveness of biological control process. This study was conducted, the natural occurrence of egg parasitoids which was assessed by the compatibility of *Trichogramma* spp. that associated with the *Helicoverpa armigera* under mixed vegetation ecosystems on-farms and on station at different agro-ecosystems in Ethiopia related with the diversity, species composition, biology, and effectiveness of recovered *Trichogramma* spp. and other egg parasitoids on *H. armigera*. The diversity of egg parasitoids was conducted on 50 randomly selected farmer's fields from four agro-ecosystems indicating that there were four main groups of Hymenopteran egg parasitoids; namely, two *Telenomus* spp (Scelionidae); two *Trichommatidae* spp. nr. *lutea* and or *armigera*, two *Trichogramma* spp nr *mwanzai* and *Trichogramma* spp. nr *bourneri* (Trochogrammatiae) and a number of unidentified species during the academic year 2014-2015. The natural field parasitism was estimated to be 33%; parasitism of the recovered egg parasitoid species according to locations varied from 27 - 40 %, while that of different crops (cotton, tomato, and pepper) also varied from 25 - 57%. Among those recovered from the surveyed agro-ecological locations, *Trichogramma* spp. nr. *bourneri* was recorded on various canopy structures of cotton, tomato and maize, including that it has a broader niche. Egg parasitoids abundance and species composition were analyzed by the Czckanowski coefficient and Shannon index indicating that *Trichogramma* spp. nr. *bourneri*, *Telenomus* spp. The *Trichogramma* spp. nr. *mwanzai* was widespread in both low (Afar) and high altitudes of agro-ecologies. The abundance of *H. armigera* eggs and its egg parasitoids in two benchmark sites at extremes of altitudes of 740 and 2024 m. a. s. l. during the main cropping season of 2014. At the lower altitudes, the highest counts of parasitized eggs were from pigeon peas where the parasitoid *Telenomus* spp. was most abundant. At higher altitudes, the highest numbers of parasitized eggs were recorded from tomatoes. The first appearance of a targeted host egg during the off-season at lower altitude was in November and it was the highest number recorded. At Guder during the off-season; *H. armigera* eggs and their parasitoids were received from November to January 2014-2015 in tomato and pigeon peas with the highest parasitism recorded in January to November, include year in tomatoes and pigeon peas with the highest parasitism recorded in January in both crops. This study concluded that the impact assessment of *H. armigera*'s natural enemies in general and egg parasitoids in particular should be done in the different localities of the country to confirm the best candidate for use as biological agent and the application of pesticides in vegetable and fiber crops based agro-ecosystems should be judiciously and for further

ecological and physiological studies with regards to *H. armigera* and its egg parasitoids interaction should be conducted. Furthermore, studies on the development of mass production techniques for effective egg parasitoids should be emphasized.

Keywords: Diversity, *Trichogramma* spp, *Helicoverpa armigera*, Egg parasitoids, Mixed vegetation ecosystem



INTRODUCTION

A community is said to have a high species diversity, if many equally or nearly abundant species are presented. High species diversity indicates a highly complex community, for a greater variety of species allow for a larger array of species interaction (Brower, 1984). Ecologists have long sought explanations for fluctuations in the abundance of insects and other animals. An attention usually tends to focus on the role of natural enemies-predators, parasites, parasitoids and pathogens (Price, 1975). Vegetation diversity influences the relative abundance of herbivore arthropods and their natural enemies, and the evolutionary responses of each component lead to complex interaction amongst them. The nature of interactions between insect pests, crops, non-host plants, and their physical environment determines the effectiveness of biological control process. The production can be broken into two components: Predators and Parasitoids were killed herbivores at higher rates in poly-culture than monoculture, and the highest mortality rates in poly-cultures significantly reduce herbivore populations.

Therefore, the effects of resource concentration and natural enemies on herbivore arthropods are complementary, but they influence mono-phagous and poly-phagous species differently (Root, 1973). A mono-phagous species is likely to be less abundant in poly-cultures than monocultures.

The agricultural production systems of Ethiopia is dominated by subsistence farmers using traditional practices that is comprised of a mixture of crops raised on patches of plots arranged in mosaic patterns (Fig. 1). This farming system creates heterogeneity and genetic diversity that can impose a physical restriction on the development of insect pests and their natural enemies. Besides, there are large scale state and private farm areas that produce cash and food crops. Such large scale farms mostly practice monoculture cropping system, which may create favorable environment for the development and dominance of a few species of insect pests, which leads to continuous application and use of different pesticides, which also had a negative impact on the existence of natural enemies and flare up of high population of the insect pests.



Figure 1. **Traditional practices and mixed farming systems at some surveyed areas of Ambo, Ethiopia (Mosaic patterns)**

The aforementioned facts have been observed in cotton producing areas of upper, middle and lower Awash rift valley regions of Ethiopia. The economically important insect pests that required high frequency of insecticide sprays was *H.armigera*, a pest of major importance in most areas where it occurs, damaging a wide variety of food, fibers, oil seeds, fodders and horticultural crops. Different control measures have been devised against *H. armigera* such as cultural methods the use of trap crops; biological control (predators, parasitoids, microbial agents and botanicals) and chemicals elsewhere in the world and in Ethiopia. However, to minimize or prevent the built-up of the insect pest, it is still important to conserve and encourage the naturally occurring natural enemies of this insect pest.

In general, research on egg parasitoids appeared to have been relatively limited in Africa simply by comparing the published research reports on egg parasitoids (Sithanantham, 2021; Southwood *et al.*, 1983). It is evident that only limited parts of Africa have been surveyed and particularly in Ethiopia the survey was only done once which was in 1973, but later in 1999 and 2001 attempts were made to detect locally occurring egg parasitoids (Mulugeta Negeri, 2005; Mulugeta Negeri and Mohammed Dawd, 2004). Hence, it is highly essential to extend the exploration for the locality occurring egg parasitoids species to different localities with widely differing ecosystems, seasons and habitats as well. The group of Chalcididae especially Trichogrammatidae which

are solely egg parasitoids also exists in the region.

The advantage of using egg parasitoids over larval parasites in biological control is that the former prevents hatching. Moreover, egg parasitoids can be mass-reared more easily; however, the success or failure in the use of egg parasitoids in biological control depends on the choice of species. The survey revealed a wide distribution of species of egg parasitoids throughout the world, especially in the genus *Trichogramma* thus indicating their importance as natural enemies of insect pests and the coexistence of more than one species of *Trichogramma* in certain area is common, while Voegelé, (1988) stated that some *Trichogramma* parasitoids may displace the other species of *Trichogramma*. This complementary research undertaken focusing on the general objective of studying the association of inter- and intra-specific interaction among *Trichogramma* spp. coexisting in mixed vegetation ecosystems parasitizing on *H. armigera* with the hypothesis that, in mixed vegetation ecosystems parasitism rates of *H. armigera* by *Trichogramma* spp. diverge and analyzed in relation to their impact on the compatibility of the different egg parasitoid species in the same ecosystems of surveyed areas.

The range of *Trichogramma* species attacking a certain host often vary depending on the habitat and/or the crop from which the host was collected (Pinto and Stouthammer, 1994) while others have been found to parasitize a host on certain crop plants but not on others (Risch *et al.*, 1983). In overall aspects, the host-habitat location, host location and host acceptance could be arbitrated by a

number of stimuli. The gross components of these interactive system could be specified as plant (=host-plant), host (=herbivorous host-insect), and enemy (=predator or parasitoids), with the assumption that the environment influences the three components independently.

In Ethiopia, there is a great potential on the use of biological agents for the control of Lepidoptera including the African bollworm. The damage on cotton production in Ethiopia is quite enormous. This insect pest takes the greatest share of pesticide spray in the cotton production areas of Ethiopia. It is estimated as many as 12 fold of spray using different chemicals is required to control it. Therefore, strategies in biological control against such economically important insect pests have to be developed and designed according to the need and limitations of the available resources. In countries such as Ethiopia, it is virtually impossible to invest on exotic natural enemies in introduction programs. The utilization of endemically available natural enemies is also limited or none. This is because of the lack of thorough knowledge of the endemic natural enemy fauna and their potential values. Especially, the search for egg parasitoids and their utility is lacking particularly in Ethiopia. Hence, the current study was to determine the performance of the diversity of native *Trichogramma* spp. and other egg parasitoids affecting *Helicoverpa armigera* in mixed vegetation ecosystems within different crop types in Ethiopia.

MATERIALS AND METHODS

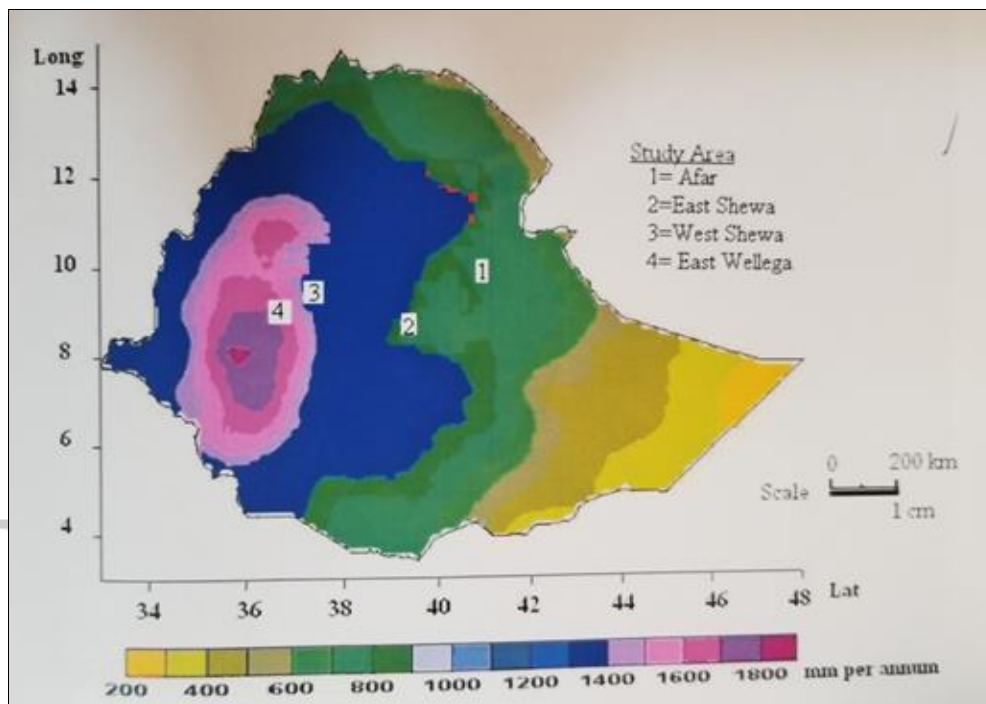
The current study was carried out on on-farms at different selected localities/sites/ and at two purposively selected benchmark sites in Ethiopia during the academic years 2014-2015.

Study Sites

On-farm

The survey study sites were randomly selected as pilot-gird survey based on the accessibility and potential of the zones and districts in targeted crops productions. Because of the large size of the regions of the country, the

survey sites were classified as central, western, eastern and northern of the administrative zones for conveniences of follow-up. Afar region was included following the Awash rift valley, the main cotton and tomato producing areas. Low, intermediate and high altitudes were also focused within the survey sites. A global positional system (GPS) was used to record the coordinates and altitudes of the particular areas. Locations of the study areas in different parts of Ethiopia and specific sampling sites within the respective locations were presented in Fig. 2.



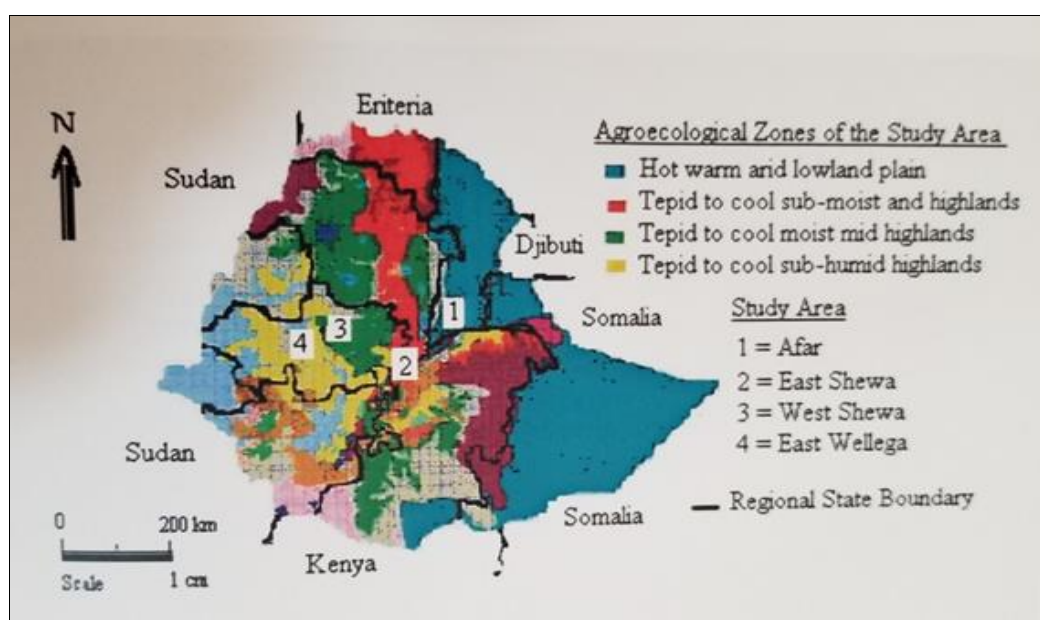


Figure 2. Locations of the study areas in different parts of Ethiopia (1= Afar;2= East Shewa; 3= West Shewa and 4 = East Wollega)

Time of the assessments

The time of on farm assessments were classified into two cropping seasons of the academic year 2014-2015 based on the typical annual rainfall calendar in Ethiopia and major altitudes and ecological regions that are illustrated under site descriptions in Tables 1 and 2. The two cropping seasons in which the assessments were conducted were main cropping seasons and off-seasons. The main cropping seasons is rainy season from May to September 2014 and the off-season which is partially the dry season October to April, 2015. Based on the

availability of the crops and growth stages assessments were carried out for three times in each cropping seasons. Rainfall in Ethiopia is generally correlated with altitudes. Middle and higher altitudes (above 1500 m.a.s.l.) receive substantially greater falls than do the lowlands, except the lowlands in the west where rainfall is high. Generally average annual rainfall of areas above 1500 meters exceeds 900mm. In the lowlands (below, 1500meters) rainfall is erratic and averages below 600mm. There is strong inter-annual variability of rainfall all over the country. The distribution of rainfall is shown in the below Table 1.

Table 1 Typical annual rainfall calendar and distributions (mm) in Ethiopia during the academic year 2014,

	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec
Dry season	+++	+++			+++					+++	+++	+++
Small rain			***	***								
Big rain						XXX	XXX	XXX	XXX			

Note= Dry season =200-400mm; Small rain =400-800mm; Big rains = 800mm < (annually)

Table 2 Major altitudes and climatic regions of Ethiopia

NN	Regions	Altitudes (meters above sea level)	Mean annual temperature (Degree centigrade)
1	Wirch (Alpine)	Above 1500	Below 10.5
2	Dega (Temperate)	3500-2500	10.5-15.5
3	Weinadega (Subtropical)	2500-1800	15.5-18.5
4	Kolla (Tropical)	1800-1500	18.5-20.0
5	Bereha (Desert)	Below 1500	Above 20.0

Source: Brehanu [1971]

Assessment for percent field parasitism

The relative importance and the percent parasitism of indigenous egg parasitoids associated with *H. armigera* were determined in some localities of Afar (rift valley areas) east Showa, west Shewa and east Wollega administrative zones of Ethiopia. From Afar, the areas mainly focused and surveyed were Tandaho, Gewane and Melkaworer. But some other localities in the surroundings such as Asayita, Metaka, Egile and Melkasadi were also assessed and eggs were collected. From east Shewa: Nuraera, Waketiyo, and Argiti (part of Arusi zone) were assessed included some nearest areas,

From west Shewa, some localities that were covered during the assessment included: Ambo; Guder: Chalia and Backotibe following the main road from Ambo to Backo. In east Wollega administrative zone the assessments carried out in the localities of Sasiga, Jima-Arjo, Guto-wayu, Sibusire, and Gobu-seyo. The main host crops focused in these areas was cotton, tomato, maize or sorghum, pepper, chick pea and Niger-seeds depending on the localities where they are growing or produced. The available eggs of the targeted insect pest were collected and the field parasitism rate were estimated

$$\% \text{ field parasitism/site} = \frac{TEP}{TEC} \times 100.$$

On - Station

Two benchmark sites were established to represent the different agro-ecologies where eight different crops serving as alternative host plants for *H.armigera* were planted. These crops were: (A). Tomato (var cal.J) 30 cm within rows; (B). Pigeon pea (short duration) 20cm within rows; (C). Sunflower (var. white Hungarian) 20 cm within rows; (D). Okra (var.Pusa sawni) 30 cm within rows; (E). Capsicum (var. Freshno) 30cm within rows; and (H). Kale (var Thousand headed) 30 cm within rows. The experimental field was arranged into blocks of four replications with the crops spaced 75cm between rows 20-40 cm within rows 20-40cm. Collections and assessment of naturally oviposited eggs and their parasitoids were conducted as follows: Examination randomly ten tagged plants along the row within each crop plot; Collection of 10-20 host eggs per crop plot; Hanging of egg cards of *H.armigera* on different parts of crop stands diagonally at three spots within the plot to trap the egg parasitoids. The relationship between host plant, host insect and egg parasitoids were noted by observing their preference and acceptability. Experiments were conducted at two benchmark sites that were established at extremely different altitudes (Melka Werer, 740 m.a.s.l and Guder/Ambo with an altitude of 1850 m.a.s.l).

Descriptions of the on-station study sites

Guder is about 12km to the west of Ambo and the benchmark site was located at the Ambo College of agricultural Experimental Station which

is 1850-2008 m.a.s.l. The total amount of annual rainfall averaged 1258.5mm with a monthly average of 104.9mm monthly minimum-maximum of 10.9mm, and 251.4mm, and the mean annual maximum, minimum and average temperatures were as recorded 27.3°C, 7.5°C and 17.4°C, respectively. Werer benchmark site was located at the altitudes of 740 m.a.s.l. in the territory of Meka Worer Agricultural Research Centre within the Afar region at the middle Awash, Amibara district, Melka-Sadi zone and located at about 285km east of Addis Ababa. The agro-ecology of the area is hot to warm humid low land. The total amount of annual rainfall averaged 590mm and with the min/maximum temperature of 26.7°C and 40.8°C.

Collection of *H. armigera* eggs and larvae

Helicoverpa armigera eggs and larvae collections were made to initiate the laboratory culture (colony) of the host insect pest and to detect the egg parasitoids naturally occurring. Eggs and larvae were collected from different farmer's fields and crops. Crops such as chick pea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativus* L.) were initially focused to obtain sufficient number of larvae. Collections were made in these two pulse crops using standard entomological net by sweeping 20 times (=one sample) in diagonal bases at six spots of the particular crop fields.

Assessment of *H. armigera* eggs and its parasitoids in various crop fields

Cotton (*Gossypium* spp)

In the experimental stations, three cotton plant stands at four different corners, totaling about 12

cotton plants per field were assessed. In large scale cotton farms, the plant stands within the quadrat (5m x 10m = 50m²) at four different spots of the field and one at the middle were considered for monitoring the abundance of the *H. armigera* (host) and its parasitoids. The plant terminals, the upper 15cm to 25cm of the crop were carefully examined including all its stems, leaves, squares and flowers for both *H. armigera* larvae and egg collections. For mass collection 50-100 eggs of *H. armigera* per field were randomly collected in bulk and kept in vials, 20 eggs each.

Chickpea (*Cicer artietrum* L) and Grass pea (*Lathyrus sativus* L.)

Host insect collections from Chickpea and Grass pea crops were conducted using two methods: Quadrat = 1m x1m and Sweeping net = 20 sweeps as one sample. The eggs were collected from the plant stands within quadrat especially at the time of assessments within chickpea fields, because the plant stand counts were not crowded within the quadrat comparing with other crop stands.

Niger seeds (*Guizotia abssinica*)

Noug (Niger seed, *Guizotia abssinica*) was assessed for larvae of *H. armigera*. The assessments were conducted during its flowering stage which is preferred by *H. armigera* larvae to feed on the seed filling flowers. The collections were made within the quadrat (1m x1m) from each plant stands by hand and kept in Petri-dish.

Sorghum (*Sorghum bicolor* L.) and Maize (*Zea mays* L.)

In row planting of maize or sorghum one plant every 5m interval within a row and 10 rows per field were

examined. The plant parts from top down, leaves (upper and lower surfaces), sorghum heads, maize cobs, silk and tassel were visually examined and the available larvae and eggs were collected.

Tomato (*Lycopersicum esculentum* Mill)

About 50-100 tomato plant stands were checked per plot depending on the size of the fields. Tomato leaves (upper and lower surface), fruits (bolls), young stems, twigs and flowers were carefully examined and the available eggs per plot were collected. Hand picking was used to collect both the larvae and eggs of *H. armigera* on tomato crops.

Pepper (*Capsicum annum* L.)

Pepper crops were also examined in different localities. The observations and collections methods used for *H. armigera* eggs and larvae within pepper crops were the same as of assessments made in the field of tomato.

Egg maintenance and removal

Later the eggs collected were carefully separated from the substrate into clean Petri-dishes using a fine and soft camel hair brush to avoid the development of mold or fungus within the vials when kept together with the fresh substrate. A maximum of 20 eggs were glued onto a piece of paper card using all purpose adhesive (UHU), sticker labeled and inserted into the middle of a vial and kept for further observations. The blackened or brown (parasitized) colored eggs were first separated from the un-parasitized eggs which were pearly white to light brown before glued into cards.

Laboratory culture establishment

Rearing of targeted host insect (*H. armigera*)

H. armigera larvae and eggs initially collected from the fields of different localities were kept under laboratory conditions in Petri-dishes. To avoid cannibalism the bigger (>2 instar) larvae were individually separated from the smaller ones. Fresh plant parts of chickpea and faba bean leaves and pods and sometimes chickpea seeds (1-2 seeds /larvae) soaked overnight (in water) were provided as food for few days. The egg masses kept glued onto paper cards placed in glass vials were incubated under laboratory conditions. *H. armigera* was also reared on modified semi-artificial diet which were prepared from soybean flour (125g), bread yeast (10g), sorbic acid (1.5g), methyl-4-hydroxybenzoate (3g), ascorbic acid (3g), casein salt (3g) and agar powder (12.5g). Forty percent formalin (2ml) of vitamin stock solution prepared following the procedure of Tackle and Jenson (1985). The larvae were reared singly in small cups (4x4.5cm) containing 10g of the larval diet and maintained at 27± 3°C and 12h (L:D) photoperiod.

Matured larvae were collected every morning and transferred to pupation cages or foam pots embedded with soil. The emerged adults were transferred in pairs into artificial rearing cages made up of wooden frames and fine mesh plastic. Adults released into the cage were monitored daily until death while the eggs were collected daily from the leaves of the tomato crops that were planted (placed) in the cage. These freshly laid eggs were provided to adult parasitoids for oviposition under

laboratory condition. The factitious hosts were reared in the plastic container consisting media. Disposable cups and small Petri-dishes with wire mesh covered were used as ovipositional arena for factitious host and for harvesting their eggs.

Rearing of egg parasitoids

The host eggs collected from the field was glued evenly between 20-30 eggs on to tracing paper card (2 x 4cm) within 1cm diameter in the centre using UHU sticker. Each of these cards was inserted into a vial and kept under laboratory condition and observed consciously for emergence of adult parasitoids and/or normal host larvae and allowed the adult parasitoids to freely move up and down and parasitized the host eggs, hence continuous. Generations of the parasitoids were maintained. The prepared diets or media, specimens, live pupae or adults of parasitoids were stored in a refrigerator. The parasitized eggs counts, parasitoids character and behavior observation were conducted using high Zoom Magnifier Stereo Microscope (SZ4045, 0.67-4x).

Detecting and initiating egg parasitoids

The adult parasitoids were recovered from *H. armigera* eggs collected from various localities and crops. The parasitized eggs were collected from tomato, cotton, maize or sorghum at the time of assessments from the localities of east Wollega, west and east Shewa and Afar. Eggs parasitized were detected by examining the color of *H. armigera* eggs. Eggs that have been attacked by *Trichogramma* and other egg parasitoids turned distinctively dark or black within three to four days after

being parasitized. Sometimes the eggs were deep grayish or brown compared to the normal eggs or the black-head eggs. Black-headed eggs were mostly three days old with the blackhead capsules of the developing host's larvae clearly visible through the egg shell, but black or brown -parasitized eggs have black egg shell that is not transparent. The last symptoms with black shell indicating the pupal stage of the parasitoids and then the fully grown wasps emerged. The black parasitized eggs could produce more than one adult wasp.

Specimen preparations for identification of egg parasitoids

The overall identification of the egg parasitoids were made based on certain characters found on the adults. The adults were used both from bulk or isolation rearing situations that were practiced at Ambo PPRC laboratory. Dead or fresh adults from the live colony were used. For specimen preparations, the adult specimens were collected and stored in 70-80% ethanol or placed directly in a clearing solution. The specimen slide mounted and observations were conducted using Zoom Stereo Microscope (SZ4045, 0.67-4X). The mountant used was Canada balsam. The adopted procedures used were: the specimens were placed in small watch glasses containing cold 10% KOH for about four hours to clear the body contents. They were transferred to distilled water to remove excess KOH; the specimens were transferred into 70% ethanol for 5 minutes and replaced the specimens through intermediate concentrations of ethanol (70% to 80% to 90%); then, the specimens transferred to a mixture of half clove oil and half 95% ethanol for several minutes and then the specimens

were transferred to 100% clove oil for 10 minutes; then a small drop of Canada balsam was placed in the center of the microscope slide and diluted with a drop of xylene when it as necessary. After that the specimen were removed from clove oil and placed in the Canada balsam on the slides and the wigs, legs and antennae were extended carefully and observation took place under microscope. The observations were made using the cover slip or without. But cover slip was placed on the top of the specimen and gentle pressure was applied from the top until the balsam reaches the cover slip edges. Finally the identification of the egg parasitoids to genus level was accomplished using the adopted keys by different citations (Nagarkati and Nagaraja, 1977; Pimm, 1991; Zerova and Fursov, 1989) as follows: *Telenmus* spp: Small yellow or brown wasp with red eyes (go to 2). Small black wasp with black eyes; *Trichogrammatoide*: Forewing with long fringe hairs. Radial vein absent; *Trichogramma* spp: Forewings with short fringe hairs Radial vein present.

In all cases, only female and males of *Trichogramma* and *Trichogrammatoides* were used in the experiments. Male parasitoids were distinguished by the un-segmented antennal flagellum with long filamentous hairs as opposed to lack of filamentous hairs in the females (Nagaraja, 1978; Pinto 1994). The normal and active adults were exposed to the factitious hosts, while the dead and unwanted adults were used in the identification. Initially all locally collected parasitoids were tentatively grouped by their body color and size. Adults having deep brown or jet black color with red eyes were labeled as

Blackish Trichogramma- SPB, while those which were grayish or yellowish with a ring or black spot on the abdomen and with red eyes were labeled as *Yellowish Trichogramma* , SPY. Other egg parasitoids recovered were black wasps with keeled abdomen, short clubbed antennae and absence of wing venations, and comparatively bigger than the *Trichogramma* species were labeled as *Telemomus* -SPT. Other unidentified eggs parasitoids recovered were red in color and distinctly different. For future confirmation of identification adults specimens of the parasitoids were kept in 95% alcohol in the repository of the biosystematics unit of the department of plant protection.

Data analysis

The data were performed using Microsoft excel and SAS (ANOVA) to test whether there were differences between the mean of different populations, based on sample taken from each localities and populations. Some common measures and estimates or diversity indexes were also used. The similarity and dissimilarity of the insect species between the quadrates within each study location (site) were estimated using Czekanowski coefficient. Species abundance has been estimated in terms of the number

of species in a defined assessed area or community which is often equated with diversity. Thus, Shannon diversity index (H+) and Equitability index (J) of egg parasitoids at different locations were performed.

RESULTS

On Farm Assessments

Percent field parasitism

The results of the assessments made in location 1(Afar) during the off-season and main cropping seasons of 2014-2015 on different crops (cotton, pepper and tomato) are summarized in Table 3. The total number of eggs of *H. armigera* were collected during the assessment was 2353 eggs. The total number of eggs of *H.armigera* collected from location 1 (Afar). Location2 east Shewa, Location 3 west Shewa and Location 4 east Wollega were 895, 443, 354, and 661 eggs, respectively. Out of the total eggs collected 771 (33%) eggs were found naturally parasitized under field conditions by different egg parasitoids. The number of eggs found parasitized at location 1 (Afar), 2 (East Shewa), 3 (West Shewa), and 4 (East Wollega) were 272, 120, 134, and 245 eggs, which means 30.4, 27.1, 37.9 and 37.1% field parasitism were realized by the naturally existing egg parasitoids.

Table 3 Field parasitism percentages within cotton, tomato and pepper crops during off-season and main cropping seasons at location 1 (Afar)

Crop/Site	Main Season			Off Season			Total		
Cotton									
	TEC	TEP	%FP/S	TEC	TEP	%FP/S	TEC	TEP	%FP/S
1	319	61	19.1	85	26	30.6	404	87	21.3
2	89	52	58.4	44	10	22.7	133	62	46.6
3	74	20	27.0	16	4	25.0	90	24	26.7
Total	482	133	104.5	145	40	78.3	627	172	94.6

Mean	160.7	44.3	34.8	48.3	13.3	26.1	209.0	57.7	31.5
Tomato									
1	15	8	53.3	29	8	27.6	44	16	36.4
2	7	3	42.9	157	52	33.1	164	55	33.5
3	17	8	47.1	2	1	50.0	19	9	47.4
Total	39	19	143.3	188	61	110.7	227	80	117.3
Mean	13.0	6.3	47.8	62.7	20.3	36.9	75.7	26.7	39.1
Pepper									
Total	29	16	55.2	12	3	25	41	19	46.3

Note: TEC= total host egg collected, TEP =total host egg parasitized, %FP/S =Percent field parasitism per site

The estimated status of natural field parasitism of the targeted host insect by different egg parasitoids during main season were 34.8% on cotton 47.8% on tomato and 5.2% on pepper, while during the off-season the parasitism status were 26.1%, 36.9%, and 25.0% on cotton, tomato and pepper, respectively (Table 3) Therefore, in location 1(Afar) the higher egg parasitism status was recorded in tomato and pepper during main season

and in tomato and cotton during off-season. In location 2 (east Shewa), the higher percentage (57.1%) of egg parasitism was recorded on cotton crops during the off-season, while the lowest parasitism (18.2%) was recorded on maize crops. The field percent parasitism records in tomato at east Shewa were 45.8% and 17.6% during main season and off-seasons, respectively (Table 4).

Table 4 Field parasitism percentages within cotton, maize and tomato crops during off-season and main cropping seasons at location 2 (east Shewa)

Crop/Site	Main Season			Off Season			Total		
Cotton									
	TEC	TEP	%FP/S	TEC	TEP	%FP/S	TEC	TEP	%FP/S
Total	91.0	21.0	23.1	14.0	8.0	57.1	105.0	29.0	27.6
Maize									
Total	10.0	2.0	20.0	11.0	2.0	18.2	21.0	4.0	19.0
Tomato									
1	20.0	11.0	55.0	64.0	10.0	15.6	84.0	21.0	25.0
2	101.0	37.0	26.6	132.0	39.0	29.5	233.0	76.0	32.5
Total	121.0	48.0	91.6	196.0	49.0	35.1	317.0	97.0	57.5

Mean	60.5	24.0	45.8	98.0	24.5	17.6	15.9	48.5	28.8
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Note: TEC= total host egg collected, TEP =total host egg parasitized, %FP/S =Percent field parasitism per site

The west Shewa zone (location 3) is situated at high to intermediate altitudes, and planted with tomato and maize only. The highest percentage parasitisms were recorded 27.2 and 40.4% on tomato in main and off-seasons. In maize, there was very minimal parasitism during main season (15%) and no parasitized eggs recorded from off-season (Table 5).

Table 5. Field parasitism percentages within cotton, maize and tomato crops during off and main seasons at location 3 (west Shewa)

Crop/Site	Main Season			Off Season			Total		
Tomato									
	TEC	TEP	%FP/S	TEC	TEP	%FP/S	TEC	TEP	%FP/S
1	17.0	4.0	23.5	185.0	75.0	40.5	202.0	79.0	39.1
2	13.0	4.0	30.8	122.0	49.0	40.2	135.0	53.0	39.3
Total	30.0	8.0	54.3	307.0	124.0	80.7	337.0	132.0	78.4
Mean	15.0	4.0	27.2	153.5	62.0	40.4	168.5	66.0	39.2
Maize									
Total	13.0	2.0	15.3	5.0	0.0	0.0	18.0	2.0	11.1
Tomato									
1	34.0	16.0	47.1	148.0	63.0	42.6	182.0	79.0	43.0
2	31.0	12.0	38.7	178.0	67.0	37.6	209.0	79.0	17.8
3	15.0	4.0	26.7	205.0	83.0	40.5	220.0	87.0	39.5
Total	80.0	32.0	112.5	531.0	213.0	120.7	611.0	245.0	120.3
Mean	26.7	10.7	37.5	177.0	71.0	40.2	204.4	81.7	40.1

Note: TEC= total host egg collected, TEP =total host egg parasitized, %FP/S =Percent field parasitism per site

From the records made in location 3 (west Shewa) and 4 (east Wollega) confirmed that the total number of eggs collected and found parasitized in the main season was low. From the current assessments, at all the observations confirmed that the existence of the targeted host insect and their egg parasitoids in spite of the

differences in agro-ecology and host crop types.

Assemblages of indigenous egg parasitoids

The initial and foremost objectives of the present study were to assess, assemble and identify the native *Trichogramma* spp. and other egg parasitoids occurring in different

locations, crop habitat and cropping seasons (off-season and main season). From on-farm assessment, a total of 771 parasitized eggs of *H. armigera* were obtained i.e., 272, 120,

134, and 245 parasitized eggs from locations 1 (Afar), 2 (east Shewa), 3 (west Shewa) and 4 (east Wollega), respectively (Table 6).

Table 6 Field parasitism (%) in all four locations assessed.

Location	Total eggs collected	Total eggs found parasitized	% field parasitism
Afar	895	272	30.4
east Shewa	443	120	27.1
west Shewa	354	134	37.9
east Wollega	661	245	37.1
Total	2353	771	132.5
Mean	588.3	192.8	33.1

These parasitized eggs were differentiated into group commonly found in all surveyed areas. From these fields collected parasitized eggs, four main groups of egg parasitoids were determined. They were: Hymenoptera: Scelionidae (2 *Telenomus spp*); Hymenoptera: *Trichogrammatidae* (2 species *Trichogrammatidae nr. lutea* and *Trichogramma nr.armigera*); Hymenoptera: *Trichogrammatidae* (2 Species: *Trichogrammatidae nr. mwanzai* and *Trichogramma nr.bournieri* and un-identified species of egg parasitoid.

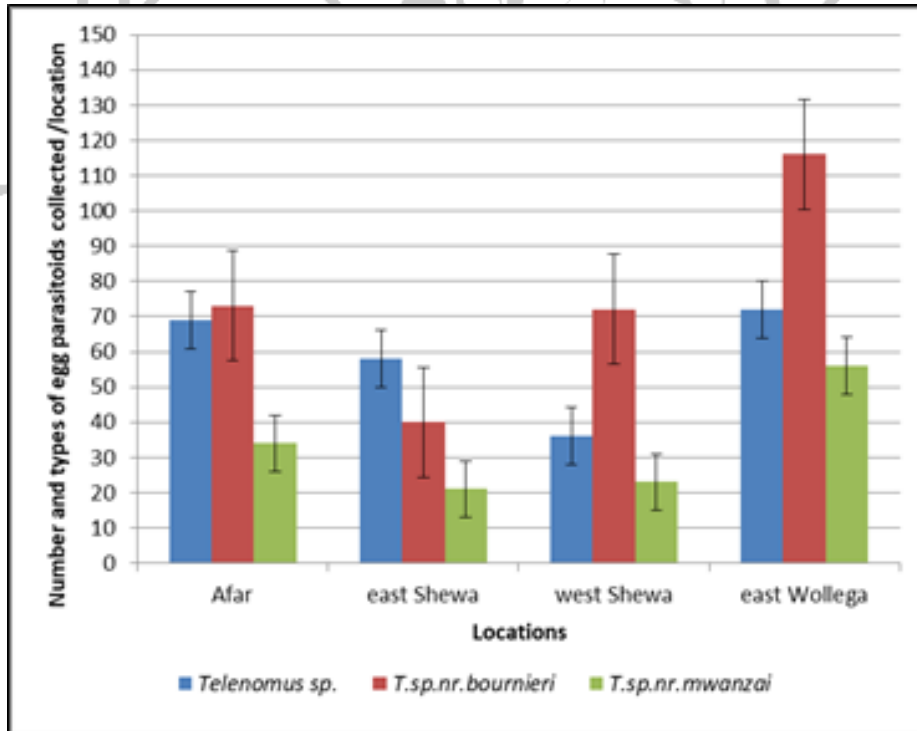
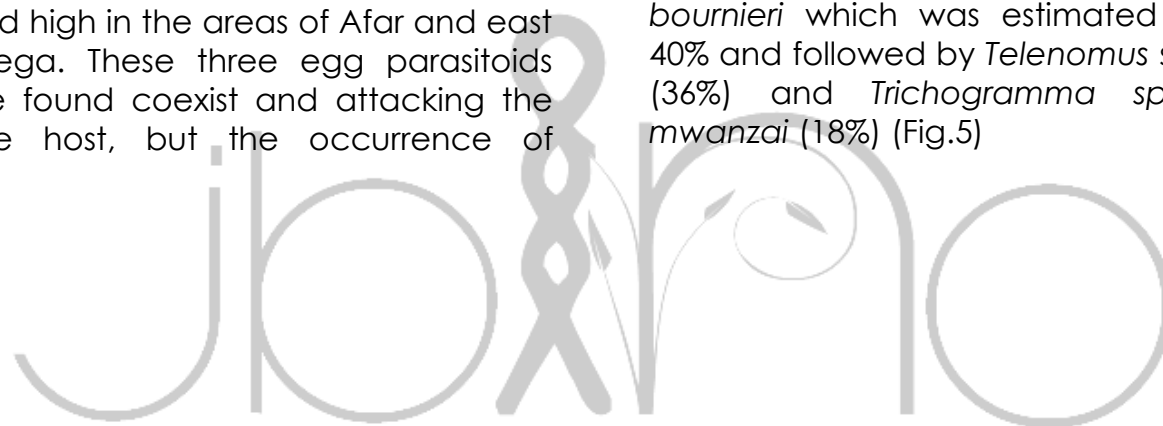


Figure 3. Total number and types of egg parasitoids recovered from *H. armigera* eggs from four different locations (*T.sp. nr. bournieri* = *Trichogramma spp. nr. bournieri*; *T.sp.nr.mwanzai* = *Trichogramma spp. nr mwanzai*)

For the future studies the following groups of parasitoids were used: the *Telenomus* spp., the dark or blackish colored *Trichogramma* grouped as *Trichogramma* spp., nr *bourneri* and the light brown or yellowish colored *Trichogramma* grouped as *Trichogramma* spp., nr. *mwanzai*. The total number and types of egg parasitoids that were recorded from the field collections of *H.armigera* eggs are summarized in Fig.3 while Fig. 4 shows their relative proportions from on-farm collections. The results indicated *Trichogramma* spp. nr. *bourneri* and *Telenomus* species are the most dominating specie of egg parasitoids recovered from all surveyed areas and found high in the areas of Afar and east Wollega. These three egg parasitoids were found coexist and attacking the same host, but the occurrence of

Trichogramma . spp. nr. *mwanzai* were less comparing to the other two egg parasitoids. Total number and types of egg parasitoids recovered from field collections of *H.armigera* eggs on different targeted crops and sites in location 1 (Afar), 2 (east Shewa), 3 (west Shewa) and 4 (east Wollega) are illustrated in Fig.3. The images of the recovered egg parasitoids that were included in the experiments are shown in Fig. 5.

From on farm collections of *H.armigera* the total relative proportion of egg parasitoids were estimated and the highest coverage seemed by *Trichogramma* spp. nr. *bourneri* which was estimated to be 40% and followed by *Telenomus* species (36%) and *Trichogramma* spp .nr. *mwanzai* (18%) (Fig.5)



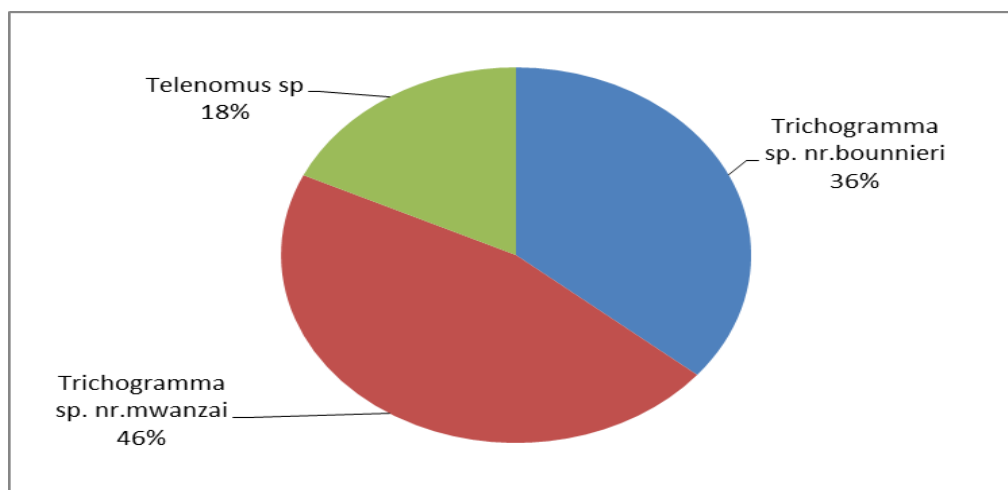


Figure 4 Relative proportion of egg parasitoids group assembled in on farm collections

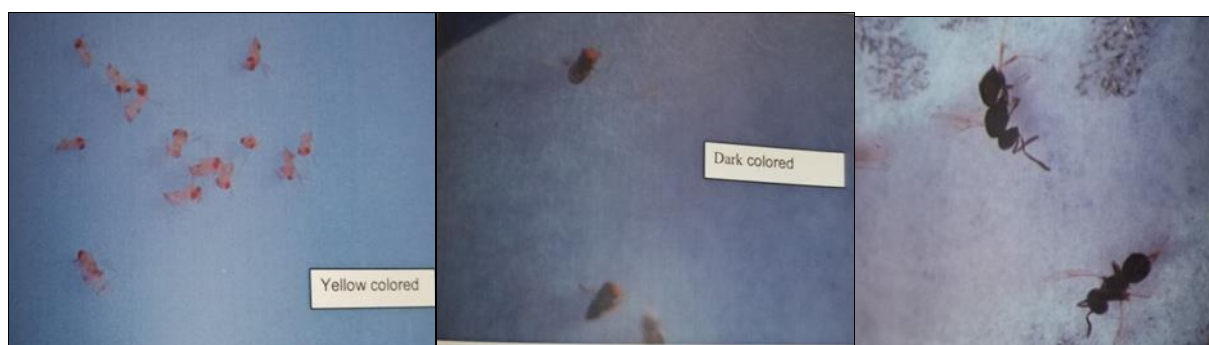


Figure 5 Types and images of parasitoids recovered

The occurrence of these egg parasitoids and their distribution were also different between two cropping seasons on different crops. The highest records of *Telenomus* species obtained from cotton and maize during off-season. In pepper and tomato seemed high during main season. *Trichogramma spp. nr. bournieri* was recorded more from tomato collection during off-season and from maize during main season. The existence and performances of egg parasitoids within different crops were varied. Within the crops such as cotton, tomato, maize and pepper the most competing egg parasitoids were *Telenomus* and *Trichogramma spp. nr. bournieri* wasps. The occurrence and distribution of these egg parasitoids were also estimated at different sites

within the survey localities. In location 1 (Afar) there were three sites assessed and the recovered egg parasitoids were recorded. At Tandaho, more eggs and their egg parasitoids collected from cotton found high during main season specifically the group of egg parasitoids that were high from *Telenomus* and *Trichogramma spp. nr. bournieri* groups. At Gewane, the records of *Telenomus* and *Trichogramma spp. nr. bournieri* were found higher during both main cropping season and off-season on cotton and tomato crops.

The assessments were conducted in some localities of location 2 (east Shewa) revealed that high parasitism rate recorded in tomato during off-season and main season at

Wake and Nura-eara on cotton and tomato during main season as well as off-season. In location 3 (west Shewa) the three species, *Telenomus*, *Trichogramma spp. nr. bournieri* and *mwanzai* were recorded from tomato within off-season at Guder and Backetibe. Number and types of egg parasitoids recovered from field collections of *H.armigera* eggs on single targeted crop (tomato) in location 4 (east Wollega) at three sites indicated that the existence of three egg parasitoids and more dominant species were *Trichogramma spp. nr. bournieri* and *Telenomus* species and their occurrences higher during off-season at each assessed sites.

recovered indigenous egg parasitoids and richness in mixed cropping patterns of the country within different focused locations and crops. Egg parasitoid species density, frequency and important values estimated in four locations, sub-sites within each location with different crop-base of 50m² at each 15 observation points are illustrated in Table 6 for both during off-season and main seasons. The density was calculated using number of individuals of each species per unit areas (750m² = 5x10x15 observation points). Each species recovered and were considered separately. Their frequency was also calculated using the proportion or percentage of sub samples which contain the species of egg parasitoids.

Attempts also made to measure the extents of the diversity of

Table 7. Egg parasitoids mean density, mean frequency and mean important values estimated by location 1(Afar), 2 (east Shewa), 3 (west Shewa) and 4 (east Wollega) on various crops assessed during main and off-season cropping.

Location	Cropping season	Recovered parasitoids	Mean density	Mean Frequency	Mean Important values
1(Afar)	Main season	<i>Telenomus spp.</i>	0.050	0.475	0.525
		<i>T.spp. nr.bournieri</i>	0.031	0.313	0.344
		<i>T. spp. nr.mwanzai</i>	0.012	0.115	0.127
	Off-season	<i>Telenomus spp.</i>	0.008	0.364	0.372
		<i>T.spp. nr.bournieri</i>	0.009	0.428	0.437
		<i>T. spp. nr.mwanzai</i>	0.004	0.207	0.211
2 (east Shewa)	Main season	<i>Telenomus spp.</i>	0.006	0.319	0.325
		<i>T.spp. nr.bournieri</i>	0.006	0.384	0.390
		<i>T. spp. nr.mwanzai</i>	0.002	0.061	0.063
	Off-season	<i>Telenomus spp.</i>	0.009	0.652	0.601
		<i>T.spp. nr.bournieri</i>	0.004	0.117	0.121
		<i>T. spp. nr.mwanzai</i>	0.002	0.011	0.033
3 (west Shewa)	Main season	<i>Telenomus spp.</i>	0.001	0.417	0.418
		<i>T.spp. nr.bournieri</i>	0.002	0.416	0.418

	Off-season	<i>T. spp. nr.mwanzai</i>	0.000	0.167	0.167
		<i>Telenomus spp.</i>	0.014	0.212	0.226
		<i>T.spp. nr.bournieri</i>	0.030	0.311	0.341
		<i>T. spp. nr.mwanzai</i>	0.010	0.144	0.154
4 (eastWollega	Main season	<i>Telenomus spp.</i>	0.006	0.601	0.607
		<i>T.spp. nr.bournieri</i>	0.004	0.268	0.272
		<i>T. spp. nr.mwanzai</i>	0.002	0.131	0.133
	Off-season	<i>Telenomus spp.</i>	0.052	0.306	0.356
		<i>T.spp. nr.bournieri</i>	0.047	0.449	0.496
		<i>T. spp. nr.mwanzai</i>	0.022	0.246	0.268

The important values, in this case taken the sum of two parameters estimated (density and frequency) for each species of egg parasitoids within assessed crops and sites only to dampens the effects of single large individuals or infrequent species. The tables illustrated how they were dense and frequently observed within the determined unit areas or quadrat at each locality, within the crop types inspected and crop seasons. It was observed *Telenomus spp.* constituted about 52.7, 32.5, 41.8 and 60.7% of the mean importance value (MIV) of the total parasitoids recovered in Afar, east Shewa, west Shewa and east Wollega, respectively, followed by the *Trichogramma spp. nr.bournieri* which gave a mean value of about 34.4, 39.0, 41.8 and 27.2% of the mean importance of the total parasitoids population recovered in the same sites respectively during the main cropping season, while the recovery of the *Trichogramma spp. nr.mwanzai* was by far less than the two species in all sites (Table 7).

Regarding the off-season recovery again the species *Telenomus* constitutes 37.2, 66.1, 22.6 and 35.6% of

the MIV of the total parasitoids recorded in Afar east Shewa, west Shewa and east Wollega, respectively, followed by *Trichogramma spp. nr. bourneiri* which constituted about 43.7, 12.1, 34.1 and 49.6% of the MIV of the total parasitoids encountered. The MIV counted for the species *Trichogramma spp. nr. mwanzai* was still low (Table. 7). In Afar region at Tandaho, the cotton based farming system during off-season and main season were tested and the Similarity ($J=0.96$) was calculated. The results showed that the Similarity and Dissimilarity of *Telenomus* and *Trichogramma spp. nr. bournieri* and *Trichogramma spp. nr mwanzai* in cotton based assessments were estimated to be 23.0 (77.0), 11.0(89) and 18.0% (82%), respectively (Table 8) and the results for tomato assessment in the same location also presented in Append. 13. At east Shewa, in cotton the similarity ($J=0.93$) were estimated 34(66); 30(70) and 38%(62%) for *Telenomus*, *Trichogramma spp. nr .bourneiri* and *Trichogramma spp. nr. mwanzia* parasitoids, respectively (Table 8). The west shewa assessments showed the similarity ($J=0.91$) of 11 % (89%) for

both *Telenomus spp.* and *Trichogramma spp. nr. bournieri*, while 8%(92%) for *Trichogramma spp. nr.mwanzai* (Table 8).

Table 8 Percent similarity and dissimilarity of egg parasitoids within cotton and tomato fields, based on the assessments made during main and off-seasons at academic years n2014-2015 at various locations in Ethiopia

Location	Crop types	Parasitoid types	Total (Off & Main season)	Similarity in %	Dissimilarity in %
Afar	Cotton	<i>Telenomus spp</i>	69	23.00	77.00
		<i>T.spp. nr. bournieri</i>	73	11.00	89.00
		<i>T.spp.nr.mwanzai</i>	34	18.00	82.00
	Tomato	<i>Telenomus spp</i>	34	12.00	88.00
		<i>T.spp.nr bournieri</i>	35	23.00	77.00
		<i>T.spp. nr. mwanzai</i>	8	25.00	75.00
East Shewa	Tomato	<i>Telenomus spp</i>	58	34.00	66.00
		<i>T.spp. nr. bournieri</i>	40	30.00	70.00
		<i>T.spp. nr. mwanzai</i>	21	38.00	62.00
West Shewa	Tomato	<i>Telenomus spp</i>	36	11.00	89.00
		<i>T.spp. nr. bournieri</i>	72	11.00	89.00
		<i>T.spp. nr.mwanzai</i>	25	8.00	92.00
East Wollega	Tomato	<i>Telenomus spp</i>	72	19.00	77.00
		<i>T.spp. nr. bournieri</i>	116	19.00	77.00
		<i>T.spp. nr.mwanzai</i>	56	14.00	86.00

The collections from east Wollega also indicated the diversity of egg parasitoid species within the tomato based farming systems. The similarity (J=0.96) and dissimilarity were 19(77); 19(77) and 14% (86%) in west Wollega for *Telenomus sp.*, *Trichogramma spp.nr.*

bournieri and *Trichogramma spp.nr.mwanzai*, respectively (Table 8), when the lower limit of the coefficient is "0" Zero it represents complete similarity and hence, the known coefficient of dissimilarity. Based on the above Dissimilarity/Similarity values the

dissimilarity of half matrices (Czekanowski coefficient) was constructed between 30 quadrates of the points of observation. Using the

above formula the diversity of egg parasitoids at Afar in cotton fields, in east Shewa, west Shewa and east Wollega in tomato field were estimated.

Table 9. Diversity of egg parasitoids at different locations (Afar, east Shewa, west Shewa and East Wollega) in Tomato based assessments.

At Afar in tomato based assessments					
Species present	Occurrence (Cover)	P_i	$\ln P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$
<i>T.sp.nr bournieri</i>	73	0.414773	-0.880025	-0.36501	0.3212218
<i>Telenomus sp.</i>	69	0.392045	-0.936377	-0.367103	0.343747
<i>T.sp.nr.mwanzia</i>	34	0.193182	-1.644123	-0.317615	0.522198
Total	176	1	-3.460526	-1.049727	1.187162
$\sum P_i \ln P_i (H') = 1.04973$					
At east Shewa in tomato based assessment					
Species present	Occurrence (Cover)	P_i	$\ln P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$
<i>T.sp.nr bournieri</i>	58	0.487395	-0.71868	-0.350281	0.25174
<i>Telenomus sp.</i>	40	0.336134	-1.090244	-0.366469	0.39954
<i>T.sp.nr.mwanzia</i>	21	0.176471	-1.734601	-0.306106	0.530972
Total	119	1	-3.543526	-1.022856	1.182252
$\sum P_i \ln P_i (H') = 1.02286$					
At west Shewa in tomato based assessment					
Species present	Occurrence (Cover)	P_i	$\ln P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$
<i>T.sp.nr bournieri</i>	72	0.541353	-0.613683	-0.332219	0.20387
<i>Telenomus sp.</i>	36	0.270677	-1.30683	-0.353728	0.462263
<i>T.sp.nr.mwanzia</i>	25	0.18797	-1.871473	-0.314187	0.525155
Total	133	1	-3.591987	-1.000135	1.191295
$\sum P_i \ln P_i (H') = 1.00013$					
At east Wollega in tomato based assessment					
Species present	Occurrence (Cover)	P_i	$\ln P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$
<i>T.sp.nr bournieri</i>	116	0.47541	-0.7443578	-0.353504	0.262858
<i>Telenomus sp.</i>	72	0.295082	-1.220502	-0.360148	0.439562
<i>T.sp.nr.mwanzia</i>	56	0.229508	-1.471817	-0.337794	0.497171
Total	244	1	-3.43897	-1051446	1.19959

$$\sum P_i \ln P_i (H') = 1.05145$$

The diversity indices (H') of different species are for Afar = 1.050, east Shewa = 1.023, west Shewa = 1.00013 and east Wollega = 1.05145. The formulas for the Shannon index commence with negative sign, but the minus signs cancel out when taking logarithms of the proportions. Any base of logarithms may be taken, with log₂ and log₁₀ being the most popular choices. The value of the index usually lies between 1.5 and 3.5 although in exceptional cases, the value can exceed 4.5. Therefore, the value of diversity index shown in the Table 10, and Table 9 indicated the existence of

Table 10 Diversity (H') and Equitability (J) index of the egg parasitoids recovered from the egg collections of different localities

Locations	Shannon diversity indices (H')	Equitability (J) index values
Afar	1.05	0.96
East Shewa	1.02	0.93
West Shewa	1.00	0.91
East Wollega	1.05	0.96

This demonstrates the manner in which evenness as well as species richness is combined in the index. The higher the values of equitability (J), the more even the species are in their distribution within the assessment quadrat. Therefore, the evenness of egg parasitoids within the assessed localities ranged in the J values between 0.91-0.96 (Table 10), this illustrating their even distributions in the surveyed localities. The dominance-diversity curves were used to demonstrate differences among the various localities in occurrence with egg parasitoids types.

high diversity of egg parasitoids in all observation sites. The evenness or equitability of the egg parasitoids were calculated using the evenness index equitability

$$J = \frac{H'}{H_{\max}} = \frac{\sum P_i \ln P_i}{\ln s}$$

Where s = the number of species; P_i = the proportion of individuals of the ith species or the abundance of the ith species expressed as proportion of total cover; ln = log base and the results obtained are presented in Table 9.

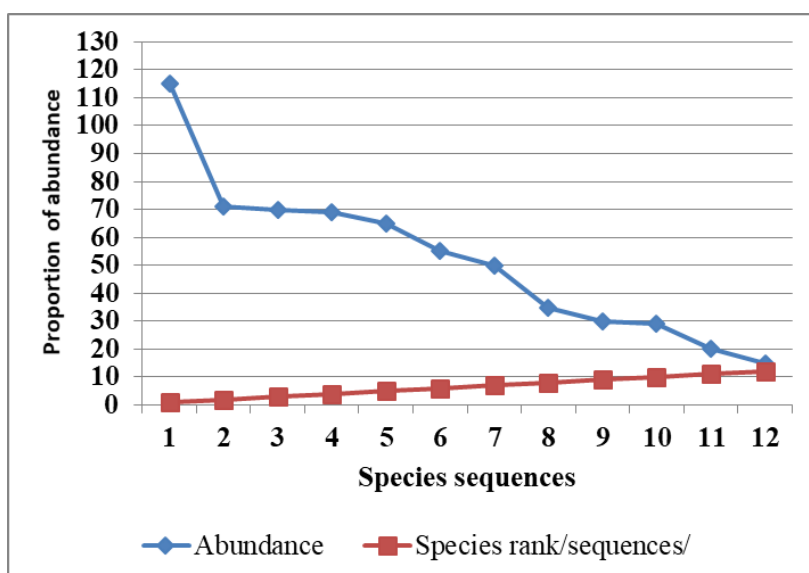


Figure 6 Dominance-diversity gradient for egg parasitoids of observation sites showing how dominance and diversity varies over location

Three types of egg parasitoids were identified in the survey. The steeper gradient indicates one or two dominant species together with a few others (Afar and east Shewa). The flatter gradient (west Shewa and east Wollega) indicates a more even distribution of species as well as a greater species richness of the localities (Fig.6). The overall egg parasitoids situations in all surveyed localities illustrated by dominance-diversity gradient was gentle, shallow and such flatter gradient indicating even distribution of the species and their richness in species compositions. The Chi square (χ^2) tests were performed showed that there were significantly different in relation to the egg parasitoids recovered from *H. arigera* eggs collected from different localities and habitats (Table 11)

Table 11 Chi square (χ^2) test for egg parasitoid species distributions in four different localities

Location (Observation sites)	Observed values	Expected	(O-E)	(O-E) ²	(O-E) ² /E	χ^2 critical =5.99, df=2, at P= 0.05
Afar						
<i>Telenous spp.</i>	106.00	87.00	19.00	361.00	4.1494NS	
<i>T.spp .nr. bourneiri</i>	114.00	87.00	27.00	729.00	8.3793**	
<i>T.spp. nr .mwanai</i>	41.00	87.00	-46	21.16	24.3218**	
East Shewa						
<i>Telenous spp.</i>	60.00	40.00	20.00	400.00	8.67**	
<i>T.spp. nr. bourneiri</i>	42.00	40.00	2.00	4.00	0.1NS	
<i>T.spp. nr. mwanzai</i>	17.00	40.00	-23.00	529.00	13.225**	
West Shewa						
<i>Telenous spp.</i>	36.00	44.33	-8.33	69.3889	1.5655NS	

<i>T.spp. nr. bourneiri</i>	72.00	44.33	27.67	765.6289	17.2711**	
<i>T.spp. nr. mwanzai</i>	25.00	44.33	-19.33	373.6489	8.4288**	
East Wollega						
<i>Telenous spp.</i>	72.00	81.33	-9.33	874.89	107.031**	
<i>T.spp. nr. bourneiri</i>	116.00	81.33	34.67	1202.009	14.7794**	
<i>T.spp. nr. mwanzai</i>	56.00	81.33	-25.33	625.00	7.684**	

It is obvious that when adult parasitoid emerges, it may or may not find itself in a habitat populated by its host. If it to survive, it must have the capacity to locate a habitat in which its host may be present. Insects use the plant or crop to provide essential resources such as feeding, mating and egg laying sites and/or refuge. In order to prolong their generations, the females even have to protect and hide their eggs or progeny from external adverse conditions and natural enemies. Therefore, discrimination during oviposition began during the first contact by checking the situation of the arena based on their own criteria, then decided to ovipositor on which chosen part of the crop. In the same manner, a parasitoid also needs to be active enough to locate the place of its hosts within the crop structure. This demonstrates directly or indirectly the compatibility of the parasitoids itself. In other way, the current thinking suggests that plant resistance works in harmony with natural enemies of insect pests, however, they are variable factors such as morphological, physiological and /or chemical characteristics of plant species which either reduce or

enhance the effectiveness of natural enemies in suppressing herbivore. In the current on-farm assessment, some important species of native egg parasitoids have been detected as potential bio-control agents. These egg parasitoids seemed to be present in all the surveyed areas as long as their hosts exist.

Results from On-stations Assessments

Parasitism Rates and Abundance of Host Eggs at both Benchmark Sites (Worer and Guder)

The assessments were conducted in these benchmark sites during the main cropping season and off-season and results obtained were presented as follows. At Worer, the total number of *H.armigera* eggs collected during off-season was 129 eggs i.e from tomato, pigeon pea, sunflower, okra and cotton crops with a total (mean) number of 39 (19.5); 30 (10); 12(6) and 32(8) eggs , respectively. The number (percent) of parasitized eggs recorded 7(18); 6(20); 4(25) and 11(34.4%) were

from tomato, pigeon pea okra and cotton crops, respectively. The egg parasitoids, *Trichogramma* spp. nr. *bourneri* parasitized for about 46.4% of the eggs that followed by *Telenomus* spp. with 39.3% and *Trichogramma* spp. nr. *mwanzai* with 17.9%. During the main cropping season assessments at Worer station, the total number of *H.armigera* eggs collected were 1169 eggs of which 40(13.3); 327(81.75); 117(23.4); 196(39.2) and 489(97.8) were from tomato, pigeon pea, sunflower, okra and cotton, respectively. The total number of eggs parasitized from the same benchmark was recorded from tomato 18(45.0%); from pigeon pea 112(34.0%); from sunflower 20(17.1%); from Okra 51(26.0%); and from cotton 100(20.4%). At Wore benchmark, the highest total parasitism rate was occurred with *Telenomus* spp at 125(41.5%) and followed by *Trichogramma* spp. nr. *bourneiri* with 114(37.8%) and *Trichogramma* spp. nr. *mwanzai* with 64 (21.3%).

At Guder benchmark site, during off-season, the total (mean) *H.armigera* eggs collected from tomato, pigeon pea, sunflower, okra, capsicum and cotton plots were 1169 eggs, i.e. 467(116.75); 433(72.17); 109(54.5); 113(56.5); 28(9.33) and 19(9.5) from tomato, pigeon pea, sunflower, okra, capsicum and cotton plots respectively. The total number of parasitized eggs counted were for about 10% (117 eggs), i.e. 68(17.0) eggs (58.1%) from tomato and 57(9.5) eggs (48.7%) from pigeon pea were

parasitized and this indicating that the highest parasitism rates were recorded on tomato and pigeon pea crops. The main egg parasitoids recovered from this site were also *Telenomus* spp, *Trichogramma* spp. nr. *bounreiri* and *Trichogramma* spp. nr. *mwanzai* with the total parasitism rate of 58.1; 57.3 and 34.2%, respectively. During the main cropping season assessments at Guder, most of the plants were with poor stands due heavy rainfall and water-log problems. However, assessments were made on the established crops such as pigeon pea okra and cotton and revealed that from a total of 211 eggs of which 13(21.8) were from pigeon pea and 429(20%) were found parasitized. The parasitism status was high in pigeon pea (62.1) and followed by cotton (21.8%).

Analysis of variance for the number of host eggs collected and parasitized indicated that there were significant differences at ($P < 0.05$) and ($P < 0.01$) in relation to their occurrence within assessed on-station (site), cropping seasons and crop types at both locations. The abundance and occurrence of *H.armigera* and their egg parasitoids were found to fluctuate between seasons, crops and locations. The *H.armigera* eggs depositions or distributions, availability of egg parasitoids and rates of parasitism seemed were more dependent upon the availability of the host crops for host insect, and /or influenced by the crop types. This indicated the egg parasitoids were more crop specific rather than

sites and seasons. Crop types were shown highly significant different ($P < 0.01$) alone and were highly significant different in the process of interactions with sites ($P < 0.01$) and seasons ($P < 0.01$) on the different parameters tested. The sites ($P = 0.67$) and seasons ($P = 0.097$) alone showed no significant differences in the availability of the host eggs, egg parasitoids and rate of parasitism. The multiple comparison analysis indicated that there were differences even among crop types grown in the locality in relation to host eggs distributions, egg parasitoids availability and rate of host eggs parasitism.

Therefore, these results revealed distinct differences observed in abundance of *H.armigera* eggs and their egg parasitoids between sites, crops and the cropping seasons. Some of the crops included in the experiment were not well established at either sites; one grew well at Worer while not at Guder. The availability of the host insect eggs were similarly variable; in most cases crops with poor vegetative growth and stunted was not preferred for oviposition by the moths which obviously preferred healthy, vigorous and young growth to lay their eggs. Crops such as pepper (*Capsicum*), particularly at Worer benchmark site was seriously infected with virus diseases and the shriveled leaves were not attractive for oviposition.

The seasonal occurrence and abundances of host insect (*H.armigera*)

eggs and their parasitoids were also considered during the assessments. The number of *H.armigera* eggs and their parasitoids recovered were computed per month throughout the year of assessments. The assessments were focused on those available crop types at each site. Albeit the limited time and logistics, efforts were focused on gathering all information necessary such that the results could give sufficient clues for further detailed investigations. At Worer specifically during off-season tomato crops produced using irrigations and also insect pests found attacking these crops and their egg parasitoids were recovered. As a result, the parasitism rates shown by *Telenomus* spp to be the most active with 3.9% and 15.4% in the months of November and December, while *Trichogramma* spp. nr. *bourneri* was recorded at 13.4% only in the month of November in which *Trichogramma* spp. nr. *mwanzai* was not found at all. On pigeon pea, very few numbers of eggs a maximum of 2 eggs collected in the month of November but not parasitized. Between the months of December and January, the number of *H.armigera* eggs record had slightly increased with the presence of all three egg parasitoids, however very few in number (17 and 11 eggs). The total parasitism rate was 21.4% with *Telenomus* spp and *Trichogramma* spp. nr. *bourneri* being 11.8% and 5.9% respectively in December and *Trichogramma* spp. nr. *mwanzai* being 27.2% in January. The occurrence of *H.armigera* eggs and its parasitoids were also recorded on okra in

November (10 eggs) and December (6 eggs). The recovered eggs with parasitoids were *Telenomus* spp. (33.3%) in November and *Trichogramma* spp. *nr. burnieri* (20.0%) in December. On cotton the availability of eggs of *H. armigera* were recovered from the month of November to February. The total number of eggs collected was 14, 10, and 5 eggs in the months of November, January and February, respectively. The parasitism rate recovered for *Telenomus* were 14.3 and 20.0% in November and January. *Trichogramma* spp. *nr. bournieri* and *Trichogramma* spp. *nr. mwanzai* parasitized 21.4% in November and 40.% in February, respectively.

During the main cropping seasons, at Worer site, the assessments were specifically conducted from May to October on different crops such as tomato, pigeon pea, sunflower okra and cotton. Other available crops in the experimental plot were also assessed but no egg was present. The availability of *H. armigera* in tomato field was recorded from the month of July through October except in the month of September. The peak numbers of *H. armigera* eggs were 32 as recorded in the month of July. In pigeon pea, the infestation of *H. armigera* was recorded from July through October and the peak number was 152 as recorded in the month of October. Other unidentified egg parasitoids were also recovered from all these collections that made in the months of July and October, 2014. From plots of sunflower,

the numbers of *H. armigera* eggs recorded from the months of June through October were 52, 18, 7, 36 and 4 eggs, respectively. On the crop of Okra 31, 56, 17, 78 and 14 eggs were collected in the months of June, July, August, September and October, respectively. Cotton plants usually found that harbored high number of host eggs in all the five months with July (122) and October (291 eggs) being the highest. The occurrence or abundance of egg parasitoids also varied from month to months and from location to location within the same crop and sites.

The other site in which similar assessments made was at Guder benchmark site. At this site both off-season and main was considered and eggs collections performed in all months of the year based on the availability of the host plants. As a result, the total number of *H. armigera* eggs collected during the off-seasons at Guder benchmark site specifically from the months of November to February on cotton were 467(116.8) and for about 35.8% and 39.4% of these eggs were collected in the months of November and January, respectively. The total parasitism status recorded in tomato field at Guder was 14.5, 11.4, 11.0, 17.9% and 15.5% in the months of November, December, January and February, respectively. The three type of egg parasitoids were also recovered from all egg collections throughout these four months of assessments. The off-season assessment on pigeon pea at Guder was carried out from the months of

November through April and the highest number of eggs were obtained in the month of January (126) and followed by November (94 eggs). The total number of eggs found parasitized was estimated at 13.2%, but ranged between 9.5-27.8% in all observational periods. The *Telenomus* species were recovered from all monthly collected eggs and its population was high in the months of November and December, while *T.spp. nr. bournieri* was more in the months of January and February. On sunflower the occurrence of *H.armigera* eggs and its egg parasitoids was recorded only in the months of January and February. Relatively higher number of eggs was parasitized (10.1%) in February with *Trichogramma spp. nr. bournieri* being recorded the highest. On Okra crops eggs were recorded in the months of February and March and the total parasitism status was estimated at 26.5%. All three species of egg parasitoids were recovered from egg collections in February, while *Telenomus spp.* was absent in the month of March. On pepper fewer number of eggs were collected and their egg parasitoids were mostly recovered in the month of February when the host's egg number (23 eggs) was the highest. Cotton did not well establish at Guder due to the higher altitude, anyhow, the presence of *H.armigera* eggs and its parasitoids were recorded in the months of February (6 eggs) and March (13eggs). The total parasitism rates were estimated for about 15.5% in the month of February for both *Telenomus* and

Trichogramma spp. nr. bournieri parasitoids.

During the main season, assessments were carried out in different crops at Guder benchmark site. On pigeon pea the assessment was conducted between the months of May to October and a number of host eggs were collected from June to September. The parasitism levels were high in the months of June, August and October. *Telenomus spp.* were recovered from the collections in May, June, July, August and October, while *Trichogramma spp. nr bournieri* from the collections of eggs in the months of June, July, August, September and October, while *Trichogramma spp. nr.mwanzai* was only recorded in the month of August. On sunflower, high numbers of eggs were collected in September, which was accounted for 61.8%. This was reflected in a high parasitism rates of 14.7% in the same month as to compare to that of recorded in October. Observations that were conducted on cotton revealed a total of 23.1% rate of parasitism at Guder during the main season. In the month of June the parasitism rate was estimate for about 15.4%, while 11.5% was recorded in July. *Telenomus* was recorded only from eggs collected in the month of July and *Trichogramma spp. nr. bournieri* recovered from the eggs that were collected every month in which a higher number was obtained in the month of June.

Discussion

An ecosystem approach towards the pest management is one approach to reducing pesticides at least at a habitat level through enhancing functional biodiversity (role of predators, parasitoids, antagonists and soil micro-fauna in security crop protection and soil fertility) in agro-ecosystems which is a key strategy to bring sustainability to production. There is a basic causal relation between biodiversity and ecosystem stability has never ended since Pimm, (1991) published his enlightening review on the topic of "the balance of nature". Antagonists of pest organisms have made numerous contributions to ecological and economical importance in agriculture and pest management. The more predatory and parasitic species and /or genotypes present in a particular landscape, the higher will be the chance that the effects of sudden environmental changes can be absorbed by ecological resilience, i.e. the ecological communities have a higher capacity to return to equilibrium population densities (Pimm, 1991). Species diversity referred as species heterogeneity, a characteristic unique to the community level of biological organization, is an expression of community structure. A community is said to have a high species diversity, if many equally or nearly abundant species are present. A survey of the natural abundance of egg parasitoids and their potential for use in biological control was reported by Hassan, (1996) and the results from 20 different countries showed that 11

genera of egg parasitoids, including 54 species occurred regularly in fields on 34 crops involving 69 insect pests.

Several plant characters interfere with the parasitoids host searching activities. The macroscopic architecture of individual plants or communities of plants have been known to influence the ability of parasitoids to locate hosts (Hassell and Waage, 1984; Price, 1991). Romeis [1997] also affirmed volatiles emitted by certain plants could arrest or repel some *Trichogramma* females, while on some plant structures eggs were hardly reachable for *Trichogramma* females as trichomes, trichome exudates and other surface chemicals negatively affect host seeking and locations. Some researchers that were conducted by ICIPE, in Kenya has shown promise for further improvements in utilizing *Trichogramma* against targeted insect pests. Research on the search egg parasitoids on Lepidopteran pests in Africa has been few, limited to 23 countries, recording 33 species of egg parasitoids occurring in four families: *Trichogrammatidae*, *Scelionidae*, *Eulophidae* and *Encyrtidae* (Sithanantham, 2001).

In Ethiopia comprises of the central high land, the marginal, tilted uplands in the west and the southern and eastern lowlands, within this broad delineation, there is a wide range of variation in altitudes and temperature. The rainfall is also variable both in periodicity (early or late rains) and on

concentration (amount of rainfall) which contribute to the unreliability of rainfall. This in turn contributes to the scarcity of stations and availability of beneficial or harmful insects and other organisms. In order to understand the spectrum and distribution of native egg parasitoids occurring in contrasting vegetables and other crops production ecologies in Ethiopia, focused on on-farm and benchmark or on station assessment being employed address their existence, diversity and performance. In understanding the importance of information on the naturally existing beneficial insects, supplementary assessments of *H. armigera* eggs abundance and their parasitoids were carried out at two benchmark sites (Guder and Worer). Mixed species cropping is often perceived as a viable tool to suppress insect pests and/or diseases. Romeis *et al.* (1997) and Sithanatham and Maniania (2000) also emphasized the importance of the impact of host plants on host range of parasitoids.

Existence and species composition

The role and population abundance of any egg parasitoids could be influenced by the habitat character and environment. The size of vegetations, distance between farm plantations and surrounding habitats could also determine their biological diversity. So if the habitat is small enough to house large number of host then eggs parasitoid has to travel out of that habitat to find host. This would make them become less effective or

compatible and abundant at one locality and not in others or vice-versa. Assessments of the diversity of egg parasitoids conducted on farmers field on randomly selected 50 sites from four contrasting agro-ecosystems indicated that there were four main groups of egg parasitoids, namely: (a) Scelionids (2 *Telenomus* spp); (b) *Trichogrammatidae*:

Trichogrammatoidea spp. *nr. lutea* and *Trichogrammatoidae* spp. *nr. armigera*), (c). *Trichogrammatidea*: *Trichogramma* spp. *nr. mwanzai* and *Trichogramma* spp. *nr. bournieri*) and d) un-identified species. Resource fragmentation, plant-host-parasitoid interactions and environmental factors may influence parasitoid diversity. Landscape effects further complicates understanding parasitoid community composition, spatial heterogeneity, movement between habitats, and ecological succession are critical process in the development of landscapes and the ecological processes within them. Relatively little is known about how landscape structure affects community interactions and diversity. Ecological studies are typically conducted within habitats and do not address emergent landscape properties such as dispersal. Clearly, to understand biodiversity within the habitat patches, landscape or regions, we must learn how species move within and between habitats in searching for resources and mates. This understanding might give certain clue how species are compatible within such patch habitats and variable landscape structures. There are general support for

a bottom-up theory that vegetation influences upper trophic levels (Rabb and Braley, 1968; Tonhasca and Byrne, 1994) and that insect species composition changes with ecological succession. Parasitoids that occur earlier in succession must be specialized to combat the toxin sequestration by herbivores while parasitoids that occur later in succession can be more generalized without the risk of sequestered toxins in the herbivores (Price, 1991). All these predictions and analysis were made to clearly indicate the complexity of factors involved in distribution, species composition or richness of insect parasitoids in any patch of habitats.

The current observation could also indicate that the differences of number of egg parasitoids availability between various location and habitat based on the factors such as landscape (agro-ecology), vegetations (availability of host plants for the host insects); climatic conditions and ability of the parasitoids to withstand any circumstances. It has been recognized for a long time that host plants can have a strong influence on level of parasitism by parasitoid Hymenoptera. The effects can occur at the host plant location, plant ages and in searching for hosts once the host plant has been located, and on the development of the parasitoid after parasitization (Steinberg *et al.*, 1992; Sithanatham and Maniana, 2000). The effects that plants have include interspecies differences, effects due physical or

chemical differences between plant strains, and even the nutritional status of a particular plant. With the agreement of this the current assessment showed distinct variations in number of host eggs, egg parasitoids and parasitism statuses on variable plant types and structures. The study results showed that the importance of *Trichogramma spp. nr. bournieri* that recovered and recorded from *H. armigera* eggs collected on cotton and tomato canopy structures inspected. This indicated that from the coexisting egg parasitoids, the *Trichogramma spp. nr. bournieri* was the most dominant and compatible having a potential role in cotton and tomato insect pest management particularly on the *H. armigera* at various agro-ecological situations. Voegelé, (1988) suggested that parasitic wasp species richness in the tropics apparently greatest at intermediate altitudes rather than at the warmer lower ones. This was also proven during the assessment that more species were recovered from the egg collections of Guder and Nekempte which was high and intermediate altitudes rather than the Afar lower altitude. The diversity indices used showed also their diversity level and suggests the detailed research required to be undertaken to properly understand the relationships between parasitoid compatibility and other ecological factors.

The abundance and occurrence of *H. armigera* and their egg parasitoids were found fluctuating

from season to season, crop to crop and location to locations. The present observations on seasonal abundance of *H. armigera* eggs, parasitism rate and type of parasitoids were undertaken under such varied climatic conditions, locations and crop types as mixed cropping systems. A total of three egg parasitoids were also obtained from the two on stations experiments. As the assessment and collections carried out constantly every week throughout the both seasons at the extreme location the occurrence were calculated on the bases of months and observed significant differences in number of host eggs, number of parasitized and type of egg parasitoids in extremely dependent on sample size and crop host specificity, egg parasitoids abundances were varied from a minimum of 5-33 individuals within the months from November-February during off-season at Guder benchmark site from variable crops. During main season in most of the cases few individual were obtained (1-6 individuals). At Worer during main season the highest records were obtained in the month of October and followed by the collection in the months of September, August, July and June while during Off-season high records were obtained only from November-December. There were considerable similarities in population of egg parasitoids and species at the two sites. Of the egg parasitoids *Telenomus spp.* and *Trichogramma spp. nr. bourneiri* were the dominant parasitoid types, followed by *Trichogrammasp.nr. mwanzai* at both benchmark sites.

Higher densities of host eggs and egg parasitoids in September, October and November 2014 might possibly be due to the higher proportion of weedy areas with various flowers of the vegetations near the observation sites. The studies on the effect of mixed species cropping systems on pest are numerous and often contradictory due to the difficulty of teasing out the ecological factors that can affect plant-insect-natural enemies relations. Andow, (1991) analyzed in 2009 studies involving 287 pest species. Compared with monocultures, the small population of insect pest in intercropping system involving 149 species which was for about 52% is a larger population that was involving only 44 species which is for about 15%. Of these 149 insect pest species were with smaller populations in intercrops 60% were monophagous while 28% were polyphagous. The population size of natural enemies of the pests was larger in the intercrop with 53% of the studies. Several authors reported that the activity of naturally occurring *Trichogramma spp* can be increased by diversifying the agro-ecosystems. Altieri *et al.*, (1993) *Trichogramma spp.*, exhibited various habitat preferences that have been recognized for quite some times. Generally, plant defense against herbivores has traditionally been studied in a bitrophic context, i.e.in predator-prey or predator-plant context. Population ecologists have been trying to explain the dynamics of insects and their natural enemies through various models in order to

provide insight into natural enemy-prey-plant interactions. Some studies showed that the insect populations tend to be regulated by the interaction of multiplicity of factors. Lawton, (1992) indicated that was not the case as it would require a million types of population dynamics; the number of types of population dynamics was probably fairly small, even if more than one type of behaviors could be found in a single species.

In this study, the resource concentration hypothesis implies that plant diversity affects herbivore densities directly, whereas in the enemy hypothesis the effects are mediated by changes in the densities of natural enemies. These have been illustrated in various experiments related to the two hypotheses that have been conducted mainly in agricultural ecosystems by comparing the abundance of herbivores and natural enemies in monocultures and poly-cultures (Andow, (1991); Romeis and Shanover, (1996) and Towns, (1962). A comprehensive literature reviewed by Andow (1991a) indicated that crop diversity reduced herbivorous insect populations and increased natural enemy densities. The host locating ability of an egg parasitoids and have effective parasitization regardless of crop types, structure and hardness conditions which can really reflect the actual performance of the egg parasitoids are not emphasized in the form of their compatibility in previous researches so far.

As far as competition and coexistence among *Trichogramma* species and other egg parasitoids are concerned in the natural systems of surveyed areas indicated that some sort of associations of two or more species of *Trichogramma* with the same host population occurs in an overlapping habitats of some species or in a particular habitat within a heterogeneous environment containing different types of habitat. From all inspected crop types at both benchmark sites, *H.armigera* eggs were found on the green parts of the productive structures depending on the type of crops may it be pods, twigs, flowers, bolls and leaves. There were variations in the distribution of eggs within the different types of crop plants, its structure and growth stages. The parasitoids, however, rated positively to increases in *H. armigera* continued parasitizing as long as the host eggs are available. In some crop types and cropping seasons or months, there was no synchrony in colonization of the crop types by both targeted insect pest and *Trichogramma* species.

Conclusion

The overall natural field parasitism was estimated to be 33% in all assessed localities. The natural field parasitism of the recovered egg parasitoid species from different locations varied from 27 to 40%. The level of field parasitism on different crops (cotton, tomato, and pepper) varied from 25 to 57%. This indicates that

identified egg parasitoids exist in varied crop and agro-ecological conditions. Overall assessment showed that the three types of egg parasitoids (*Telenomus*, *Trichogramma spp. nr. bourneiri* and *Trichogramma spp. nr. mwanzai*) were present in all assessed localities (low to high altitudes) even though *Trichogramma spp. nr. mwanzai* was comparatively lesser in number at all locations. The other two egg parasitoids (*Telenomus* and *Trichogramma spp. nr. bourneri*) were the most abundant, widely distributed and effective native egg parasitoids of *H. armigera* under field conditions. The significance of the study implies, in addition to testing some ecological hypothesis that it has contributed a considerable amount of fundamental information on the occurrence of native egg parasitoids, their distribution, abundance, species composition and performance. The study has also determined the potential native egg parasitoid types that went important components biological control agents against *H.armigera*.

This study has shown that among the recorded egg parasitoid species, the *Trichogramma spp. nr. bourneri* was the most dominant on host eggs collected from both cotton and tomato canopy structures. As such, when compared with other coexisting egg parasitoids, *Trichogramma spp. nr. bourneiri* has proven to be the most compatible and the having the best potential role in suppressing targeted host egg within the cotton and tomato

canopy structure. The overall egg parasitoids situations in all surveyed localities as illustrated by the dominance-diversity gradient was gentle, shallow and flatter thus indicating an even distribution of the species and their richness in species composition. The results indicated the dominant species in all surveyed areas were *Trichogramma spp. nr. bourneri* and *Telenomus spp.* followed by *Trichogramma spp. nr. mwanzai*. From these results it is possible to understand that a number of factors could control egg parasitoids species compositions and diversity, but mainly management status is one of the most critical in the course of diversification and intensification of agricultural ecosystems. Thus, in terms of management and conservation goals careful consideration has to be given to local environment and to the management strategies. This study could be recommended that impact assessment of *H. armigera*'s natural enemies in general and egg parasitoids in particular should be done in the different localities of the country to confirm the best candidate for use as biological agents and a awareness should be created among the beneficiaries with regard to *H. armigera* egg parasitoids in vegetable and cotton based agro-ecosystems. Further ecological and physiological studies with regards to *H.armigera* and its egg parasitoids interaction should be conducted.

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