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Impact of parasitoids on immature stages (Larva and pupa) of *Plutella xylostella*

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Abstract
The studies concerning the population density of *Plutella xylostella* and percent parasitism by various larval and pupal parasitoids in cabbage crop. The meteorological data of Taxila site was obtained from metrological center of Arid Agriculture Rawalpindi in 2012. The result related to larval and pupal population density initiated with (1.70) larvae/plant and (0.5) pupae/plant was recorded with average minimum temperature and maximum temperature (18.92°C and 34.10°C) at 40th of calendar week of October progressively. Highest larval and pupal density 11.40 ± 1.7/plant and 4.8/plant were recorded with minimum temperature and maximum temperature 11°C and 27.43°C respectively. The first parasitism 8%, 8% and 4% were recorded on *Diadegma insulare*, *Oomyzus sokolowskii* and *Dinoptera collaris* where the minimum temperature and maximum temperature were 18.14 °C and 32.64°C at 41st week of calendar. Highest parasitism level 16% and 12% were recorded by *O. sokolowskii* and *D. collaris* at 43rd calendar week and on *D. insulare* the 48% parasitism level was recorded. The effect of early and late stage of cabbage crop on larvae and pupae of *P. xylostella* 5.43 larvae/plant and 6.55 larvae/plant and 2.24 pupae/plant and 2.76 pupae/plant were observed during 2012. The parasitism level by parasitoids, the parasitism level 20%, 8% and 6.4% was recorded on early stage of cabbage crop by *D. insulare*, *O. sokolowskii* while, parasitism 16%, 2% and 4% on late stage were recorded, respectively.

Keywords: cabbage, parasitism, *Plutella xylostella*

1. Introduction
Plutella xylostella is the most destructive insect pest of cabbage crop and having potential to adopt various climatic conditions. It also has great economic importance as pest of cruciferous plants throughout the world [1]. This pest greatly affects the yield and the quality of vegetables due to foliage feeding and reaction of consumer residues of the caterpillar in marketed vegetable [2]. *P. xylostella* can cause severe economic losses when it is not managed.
Population densities of insect pest can be regulated by various management practices along with naturally occurring biocontrol agents. It has been suggested by Talekar and Shelton, 1993 [3] that parasitoids might become the most effective factors which can control this pest in near future. All life stages of *P. xylostella* have various natural enemies but larval parasitoids are most dominant and effective in suppressing the pest populations [4, 5].
D. insulare is the very important parasitoid of *P. xylostella* in South Carolina and North America [6, 7, 8] studied the population density of larval and pupal parasitism of *P. xylostella* by *D. insulare* in South Carolina. As major larval parasitoid of *P. xylostella*, parasitism level (58.5%) was recorded. *O. sokolowskii* is the other very important and major parasitoid of *P. xylostella* found mostly in Asian countries including India and Pakistan [9, 10]. *O. sokolowskii* belongs to the family Eulophidae (Hymenoptera) and its parasitism started later in the season as compared to the parasitism by *D. insulare*, usually when *P. xylostella* population was at or above economic threshold level. Parasitism level 0 to 7.3% has been recorded of *O. sokolowskii*, *D. insulare* and *O. sokolowskii which* had remained inadequate to maintain *P. xylostella* populations below economic threshold level in commercial collard fields in South Carolina [8].
As endo-parasitoid, *D. insulare* parasitized the larvae of *P. xylostella* and its adult emerges from pre-pupa soon after *P. xylostella* forms its cocoon [8]. Larvae of *D. insulare* make its own cocoon inside the host and push the remains to bottom of *P. xylostella* cocoon [11]. *D. insulare* is most suitable for IPM programs against *P. xylostella* due to its synchronization with host's developing stages and outstanding searching ability [11].

Integrated pest management techniques were developed by Clemson University (South Carolina) which integrated all techniques such as pest investigation, cultural practices, use of natural enemies and creating the favorable conditions to enhance the efficiency of biocontrol agents of *P. xylostella* [8]. Field studies on the influence of various parasitoids attacking on particular pest are helpful to select effective parasitoids. IPM programs play vital role to conserve natural enemies through different practices such as inundative and inoculative release [12]. From Mexico, [13] McCully, 1992 identified *D. insulare* as the most efficient parasitoid of *P. xylostella* but some other species have also been recorded as parasitoids of this insect pest. More work is needed to be done to develop an effective integrated management strategy for *P. xylostella*.

This study was conducted to determine the larval and pupal population densities and percent parasitism of *P. xylostella* by *D. insulare* in untreated crucifier fields. As host plant genotype has an effect on the herbivore insect pest and the parasitoids living in/ on the insect pest [14]. Present study was designed to examine the impact of early and late stage of host plant (Golden acre variety of cabbage) on *P. xylostella* population build up and its rate of parasitism by various parasitoids.

2. Materials and Methods

This study was carried out on cabbage crop at a farmers' fields, near Taxila in October-December (2012). At experimental sites, cabbage fields of 22m width and 20m length was selected within commercial fields transplanted through 4-week old cabbage cultivar "Golden acre" seedlings. Plant spacing was 30cm within rows and 0.9m in width. Four plots, each 1.8m (two rows) in width and 4.5m in length were selected and demarcated with flags.

P. xylostella larvae and pupae were counted but not removed, on five randomly selected plants from each of the selected four plots weekly until harvest. Host density was determined as the mean number of *P. xylostella* larvae and pupae per cabbage plant. Fourth instars *P. xylostella* larvae and pupae were collected weekly from cabbage plants within experimental sites, other than from four selected plots. Lower numbers (25) of *P. xylostella* larvae and pupae were collected during early crop growth and relatively higher numbers if population of *P. xylostella* increased were collected during later stage of crop growth. Larvae and pupae were collected in plastic containers in groups of approximately 25 and were stored in a cooler with ice during collection and transportation to the laboratory.

P. xylostella larvae and pupae were carried to the laboratory and reared at 27±2°C and 70±10% relative humidity, with a photoperiod of 16:8 (Light and Dark) hours. *P. xylostella* larvae were placed in cages and fed fresh cabbage leaves daily until pupation. *P. xylostella* pupae were collected as cocoons which were attached to leaves. Leaf sections (3×3cm) with one pupa each, were cut and placed individually into small petri dishes and kept in the rearing room. Petri dishes were checked on daily basis and adults of *P. xylostella* and all parasitoids emergences were recorded while, *P. xylostella* larval and pupal mortality was documented. The voucher specimens of parasitoids were kept in insect box to be placed in the Insect Museum at Biosystematics Laboratory of the Department of Entomology. Percent parasitism of *P. xylostella* by larval and pupal parasitoids were calculated using the formula [(number of the parasitoid adults = A) / (number of adult insects (*P. xylostella* + A + other parasitoids) that emerged)] × 100.

2.1 Statistical Analysis

Percent parasitism of each sampling period was calculated by dividing total number of parasitoids with the number of emerged parasitoids and *P. xylostella*. Mean insect number per plant was calculated for each stage (larvae and pupae) of insect. Population density, percent parasitism level and weather data of both stages of cabbage crop (early and late stage) was compared by using statistical analysis of variance (ANOVA) using MS Excel version 2007.

3. Results

3.1 Dynamics of *P. xylostella* Larval and Pupal Density on Cabbage Crop with Maximum and Minimum Temperature in Taxila Site

3.1.1 Larval population density of *P. xylostella*

Figure (1) is elaborating the larval and pupal density/plant on cabbage crop with maximum and minimum temperature at Taxila in autumn season during 2012. Regarding larval population density, results showed that it initiated with 1.70±0.65 larvae/plant at 40th calendar week in October when average minimum and maximum temperature (18.92°C and 34.10°C) was observed, respectively. After that larval population density increased progressively and reached to 10.25±1.60 larvae/plant during 43th calendar week in October, where the average minimum and maximum temperature was 12°C and 27.71°C, respectively. During 45th calendar week in November, highest population density (11.40±1.7 larvae/plant) was recorded, when the average minimum and maximum temperature (11°C and 27.43°C) was observed, respectively. Ultimately population density (2.15±0.73 larvae/plant) was observed during last week of season and average minimum and maximum temperature (6.93°C and 22.17°C) was recorded, respectively.

Results showed that higher population was observed during October on cabbage plant, while lowest population (*P. xylostella)* was recorded during December on cultivated cabbage crop.

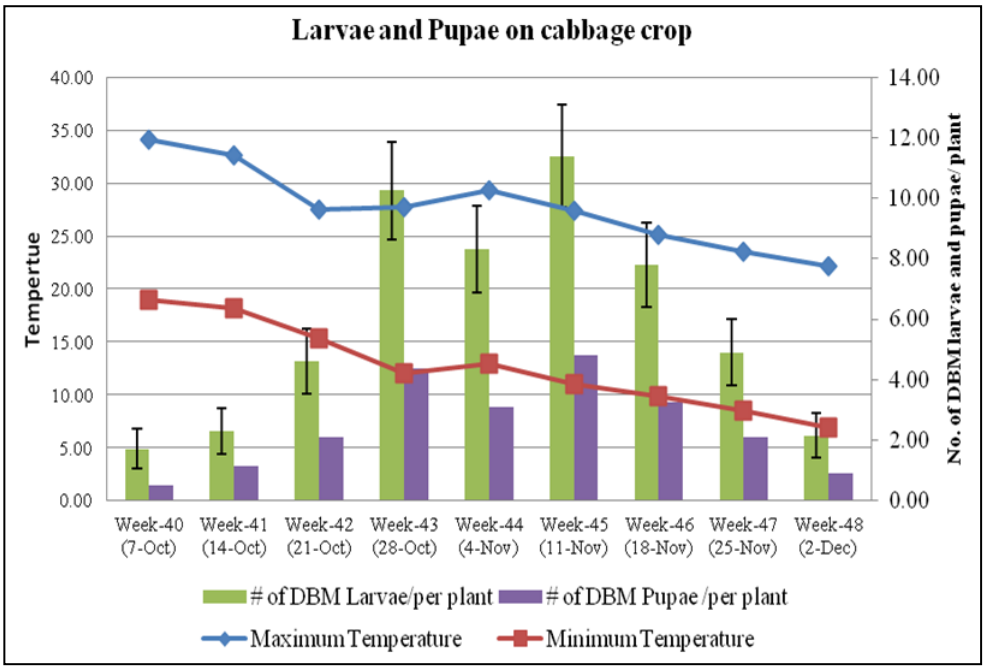

Fig 1: Mean larval and pupal density of *P. xylostella* on cabbage crop with maximum and minimum temperature in autumn season during 2012, at Taxila site.

3.1.2 Pupal population density of *P. xylostella*

Regarding pupal population density, figure (1) showed that it initiated with 0.5 pupae/plant at 40th calendar week in October when average minimum and maximum temperature was 18.92°C and 34.10°C, respectively. After that pupal population density increased progressively and reached to 4.35 pupae/ plant during 43th calendar week in October, when average minimum and maximum temperature was 12°C and 27.71°C, respectively afterwards population density (3.1 pupae/plant) declined. During 45th calendar week in November, highest population density (4.8 pupae/plant) was recorded, when average minimum and maximum temperature (11°C and 27.43°C) was observed, respectively. Ultimately population density of 0.9 pupae/plant was observed during last week of season, when average minimum and maximum temperature was 6.93°C and 22.17°C, respectively.

3.1.3 Percent parasitism by *D. insulare*

D. insulare showed zero percent parasitism in the 40th calendar week; whereas 8% percent parasitism was recorded during 41st week of calendar (October) when minimum and maximum temperature was 18.14°C and 32.64°C, respectively. Then, progressive increase in the percent parasitism was observed and it reached to the highest point (48%) during 44th calendar week (November), when minimum and maximum temperatures were recorded (12.93°C and 29.29°C),

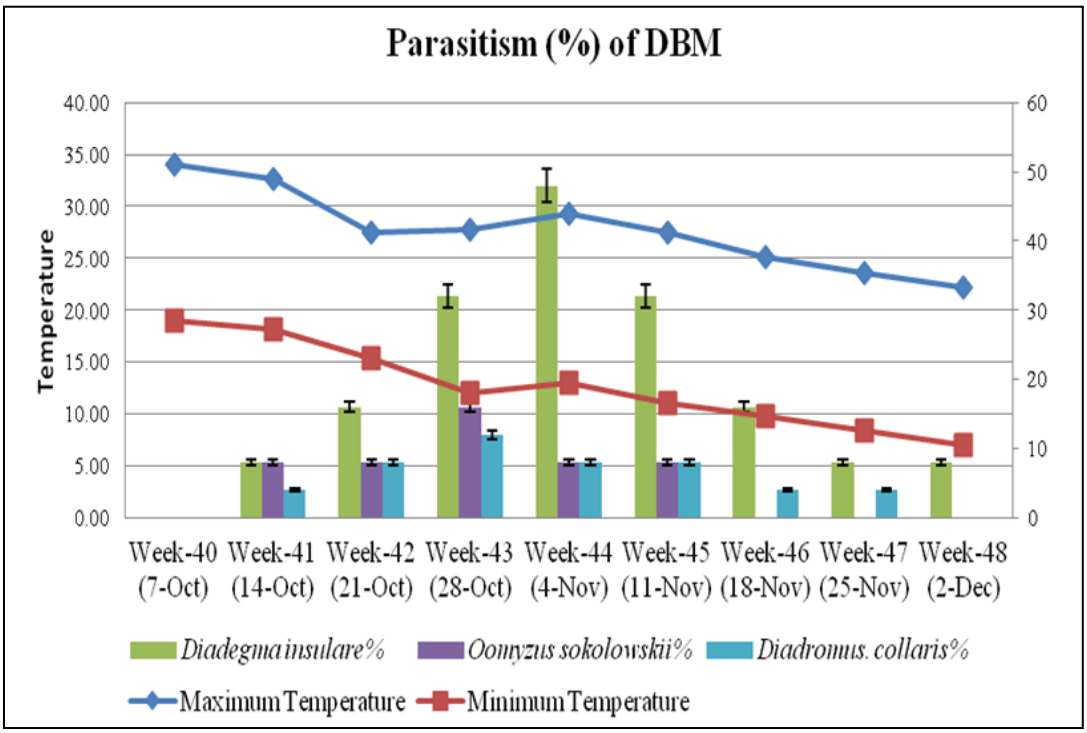

Fig 2: Impact of parasitoids on immature stages (larvae and pupae) of *P. xylostella* in cabbage field during autumn season (2012) in Taxila site respectively.

After that a gradual decrease in percent parasitism was observed, which reached to 8% during 48th calendar week, when minimum and maximum temperatures (6.93°C and 22.17°C) were recorded, respectively.

3.1.4 Percent parasitism by *O. sokolowskii*

Percent parasitism by *O. sokolowskii* was zero during 40th calendar week; whereas 8% percent parasitism was observed during 41st week of calendar (October) when, the minimum and maximum temperature was 8.14°C and 32.64°C, respectively. Then, a progressive increase in the percent parasitism was calculated and it reached to the highest point was (16%) during 43rd calendar week (October), during which the minimum and maximum temperature was 12.00°C and 27.71°C, respectively. After that a gradual decrease in percent parasitism was observed and noted to be 8% during 44th week in November, when minimum and maximum temperature was 12.93°C and 29.29°C, respectively. During 45th week percent parasitism remained the same (8%) but after that zero parasitism was observed in the last three weeks.

3.1.5 Percent parasitism by *D. collaris*

Percent parasitism was zero in case of by *D. collaris* during 40th calendar week, whereas 4% percent parasitism was recorded during 41st week of calendar (October) when minimum and maximum temperature was 18.14°C and 32.64°C, respectively. Then a progressive increase in the percent parasitism was recorded and it reached to the highest point (12%) during 43rd calendar week (October), when minimum and maximum temperature was12.00°C and 27.71°C, respectively. During 47th week percent parasitism remained same (4%) but later no parasitism was observed in last week.

3.1.6 Effect of Early and Late Stage Cabbage Crop on *P. xylostella* Larvae and Pupae

The effects of early and late stages of cabbage crop on *P. xylostella* larvae and pupae per plant were monitored as shown in figure (3). The mean larval density (5.43 and 6.55/plant) was observed at early and late stages of cabbage crop, respectively. Similarly, mean number (2.24 and 2.76/plant) of pupae per plant at early stage and late stage of cabbage crop was recorded, respectively. These figures demonstrated graphical differences between populations at early and late stage of cabbage crop but statistically no significant differences were observed among crop stages. Thus, it is concluded that crop stage (early or late) may not have any significant impact on the population buildup of *P. xylostella* larvae and pupae.

3.1.7 Effect of Early and Late Stage Cabbage Crop on Parasitoid Species

The effects of early and late stage of cabbage crop on percent parasitism by three parasitoid species i.e. *D. insulare*, *O. sokolowskii* and *D. collaris* were monitored in autumn season during 2012 at Taxila, as shown in figure (4). Mean percent parasitism (20.8%, 8% and 6.4%) of *P. xylostella* at early stage of cabbage crop was recorded by *D. insulare*, *O. sokolowskii* and *D. collaris*, respectively. While, at late stage of cabbage crop percent parasitism (16%, 2% and 4%) was recorded by *D. insulare*, *O. sokolowskii* and *D. collaris*, respectively. These figures elaborates graphical differences between percent parasitism at early and late stage of cabbage crop but statistically no significant differences were observed among crop stages. Thus, it was concluded that the impact of crop stage (early and late) didn't had a significant impact on percent parasitism for any of the parasitoids species which was likely to be passed on through *P. xylostella* larvae feeding on cabbage crop.

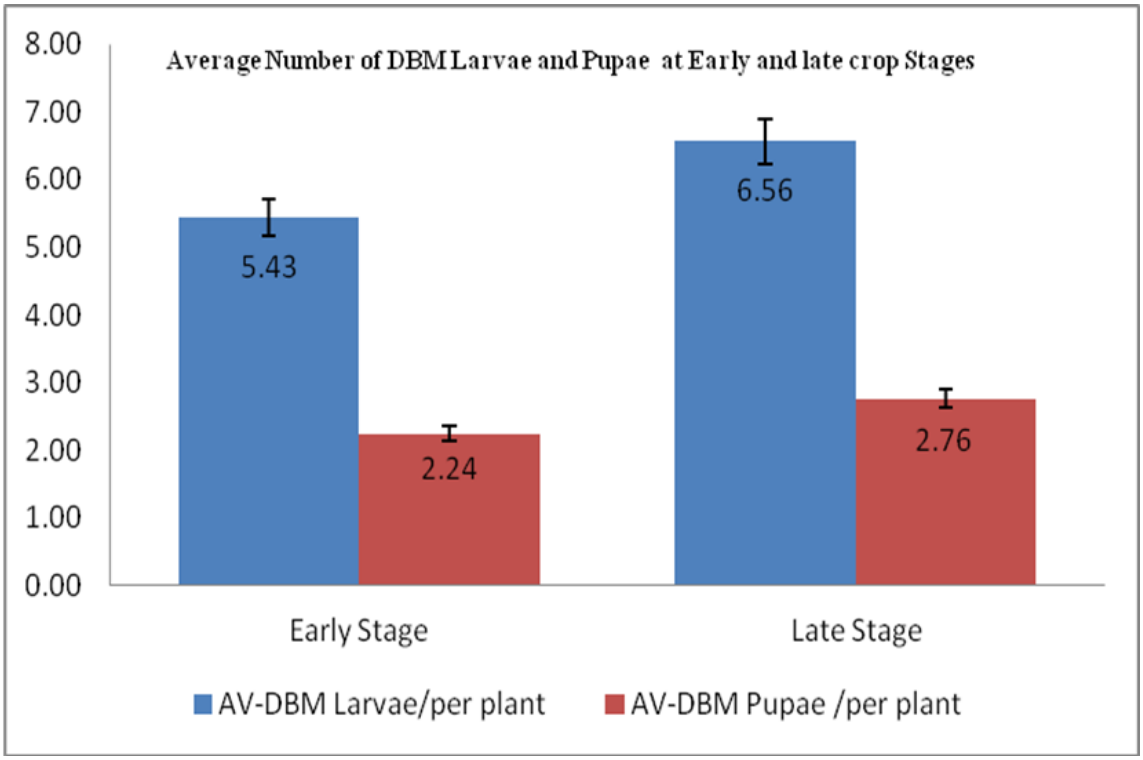

Fig 3: Average number of *P. xylostella* larvae and pupae at early and late stages of cabbage crop in autumn season during 2012 at Taxila site

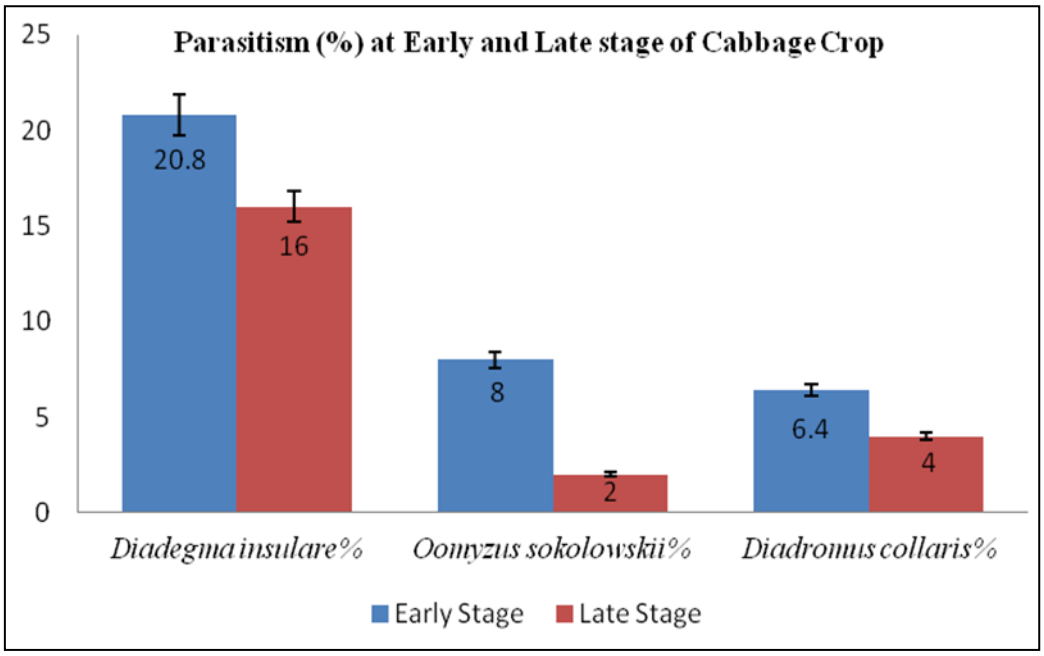

Fig 4: Percent parasitism by three parasitoid species at early and late stages of cabbage crop in autumn season during 2012 at Taxila site.

3.2 Comparison of Early and Late Stage of Cabbage Crop on *P. xylostella* (Larvae, Pupae) and Parasitoids (*D. insulare, O. sokolowskii* and *D. collaris*)

Statistical analysis regarding mean *P. xylostella* larvae and pupae per plant showed that early and late stage of cabbage crop had no significant effect formally, so null hypothesis is accepted. Similarly, percent parasitism level by *D. insulare*, *O. sokolowskii* and *D. collaris* was not significantly different statistically, thus null hypothesis was accepted. It showed that percent parasitism by concerned parasitoids was not affected by early and late stage of cabbage crop. Data obtained during autumn season (2012) at Taxila is shown in Table (1).

Results of this experiment described *P. xylostella* larval and pupal densities which were monitored/counted on cabbage crop in autumn season during 2012. Population of *P. xylostella* (larvae and pupae) started building-up from 7th to 28th October and then declined on 4th November. This decline might be due to the effect of environmental factors and parasitism of parasitoid species. Suddenly, the population density was reached to highest point (11.40±1.69/plant) due to favorable environmental conditions (average min. and max. temperature 11.0°C and 27.43°C, respectively). During last three meteorological weeks (46th to 48th) in November and December, larval and pupal population density of *P. xylostella* declined gradually due to decrease in minimum temperature (9.83°C to 6.93°C) and maximum temperature (25.11°C to 22.17°C), which effected unfavorably the development of *P. xylostella* population.

Table 1: Comparison of early and late stage of cabbage, *P. xylostella* larvae, pupae and parasitism level by parasitoids

Compared	Mean ± SE	F_{cal}	F_{cri}	Result
P. xylostella Larvae/ plant	5.43^{E}, 6.56^{L}, ± 3.34^{E}, 3.96^{L}	0.193	5.60	A NH
P. xylostella Pupae/ plant	2.24^{E}, 2.76^{L}, ± 236^{E}, 2.77^{L}	0.239	5.60	ANH
D. insulare %	20.8^{E},16.00^{L},±19.27^{E},11.3^{L}	0.192	5.60	ANH
O.sokolowskii%	8.00^{E}, 2.00^{L}, ± 5.66^{E}, 4.00^{L}	3.18	5.60	ANH
D. collaris %	6.4^{E}, 4.00^{L}, ± 4.56^{E}, 3.27^{L}	0.78	5.60	ANH

Note: E stands for early stage and L stands for late stage of crop

4. Discussion

Data concerning the dynamics of percent parasitism showed that initially (during 40th week) no parasitism was observed due to unfavorable meteorological conditions for all three parasitoids. During next week's 41st till 44th, percent parasitism started building up and gradually increased to the peak (48%) by *D. insulare* but for *O. sokolowskii* and *D. collaris* highest percent parasitism level was 16% and 12% respectively during 43rd week due to environmental conditions and the competition between the parasitoids. It was observed that *D. insulare* was the most dominant larval parasitoid with highest parasitism level as compared to other larval-pupal and pupal parasitoids. Highest parasitism level by *D. insulare* was reported by Martinez-Castillo *et al*., 2002 [12] which might be due to the low population of larvae and pupae (*P. xylostella*) and favorable weather conditions.

Waage and Cherry, 1992 [15] reported the potential parasitism level and population increase in *O. sokolowskii*, when maximum temperatures (32°C-35°C) were recorded. He also reported that the optimum temperature ranging from 32°C-35°C are favorable environment for survival and reproduction.

Bahar *et al*., 2012 [16] reported that overall percent parasitism was decreased due to the mortality of pupal stage, while parasitism level (67%) was recorded, when temperature was at 22ºC and fluctuation in temperature was 7ºC whereas, lowest percent parasitism (30%) was recorded at 30ºC.

After that overall percent parasitism was decreased and reached to minimum during 48th week. It might be noted that for *D. insulare* optimum minimum and maximum temperature were found to be 12.93°C and 29.29°C while for *O. sokolowskii* and *D. collaris* 12°C and 27.71°C were favorable minimum and maximum temperature, respectively. Furthermore, results elaborated that *D. insulare* was observed to be most effective among the three studied parasitoids. This might be due to the competition between larval and pupal

parasitoids of *P. xylostella* and also the environmental conditions of the regions might had affected the parasitism level of monitored parasitoid species. Findings of the effect of cabbage crop stages on *P. xylostella* larval and pupal population elaborated that maximum number of *P. xylostella* density was observed at late crop stage as compared to early stage. It was suggested that late stage of cabbage crop was more susceptible to build up *P. xylostella* (larvae and pupae) population. This may be due to cabbage crop (early stage) produced volatiles were unfavorable for the parasitism of *P. xylostella* by parasitoids species.

According to Ayalew, 2006 [17] highest population of larvae and pupae on late stage of cabbage crop was recorded during 3rd week to last week in March, because the population was initiated during 1st week to 2nd week in February/April could be estimated when cabbage crop transplanted in December. This might be due to crop volatility and favorable weather conditions for pest. Natural enemies of herbivorous insects might utilize the plant volatile phytochemicals released during feeding and produced from larval feeding, which indicated their hosts [18].

Similarly, the influence of cabbage crop stages on the parasitoid species elaborated that maximum percent parasitism was observed at early stage of cabbage crop productive growth as compared to late stage. This may be due to cabbage crop (early stage) produced volatiles were unfavorable for the parasitism of *P. xylostella* by parasitoids species. According to Ayalew, 2006 [17] parasitoids of *P. xylostella* parasitized larvae and pupae at different percentage on different stages of cabbage. The highest parasitism level (35.2%) was recorded on early stage (cabbage crop) when minimum population of immature larvae and pupae (*P. xylostella*) was recorded. Analysis of variance (ANOVA) showed non-significant effect of cabbage crop growth stages on larvae and pupae (*P. xylostella*) population densities and percent parasitism by parasitoid species.

5. Conclusion

It is concluded that the maximum pest density was observed on late crop stages. It is suggested that late stage of cabbage crop was more susceptible to build up *P. xylostella* (larvae and pupae) population.

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