Host Preference and Abundance of Cucumber Moth (*Diaphania indica*), A Potential Threat to Cucurbitaceous Vegetables in Bangladesh

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Abstract. Many pests are known to damage cucurbitaceous vegetables in Bangladesh but the abundance and host preference of the cucumber moth, *Diaphania indica* (Crambidae: Lepidoptera) has not been studied previously. Efforts were made to study biology, natural abundance and extent of damage and the influence of seasonal parameters on *D. indica*. The life cycle duration of *D. indica* was 17.4 ± 0.36 days, where the incubation period, larval period and adult longevity were 3.5 ± 0.16, 12.4 ± 0.16 and 3.5 ± 0.16 days respectively. Their abundance was high in the summer season and positively correlated with the temperature increases. Snake gourd followed by ridge gourd was the preferred and bitter gourd was the less preferred summer vegetable. In contrast, significantly lower leaf infestations and larval presence were observed in the winter vegetables. The findings extrapolate the potential of *D. indica* as a threat to summer cucurbitaceous vegetables in the future.

Keywords: incidence; seasonal abundance; *Diaphania indica*; cucumber moth

Introduction

Many insect pests have been identified and quantified as economic damaging pests of cucurbitaceous vegetables in Bangladesh (Sultana *et al*., 2017; Rahman and Uddin, 2016; Asafuddaullah *et al*., 2015; Rahman *et al*., 2015; Kamal *et al*., 2014). Cucurbitaceous vegetables occupy a major portion of the vegetable market in the summer season in Bangladesh. Pests like the cucurbit fruit fly, red pumpkin beetle, Epilachna beetles are the most common pests of cucurbitaceous vegetables regardless of seasons and regions of the country (Rahman and Uddin, 2016; Asafuddaullah *et al*., 2015; Rahman *et al*., 2015; Kamal *et al*., 2013). On the other hand, the cucumber moth, *Diaphania indica* (Crambidae: Lepidoptera) can be found on cucurbitaceous vegetables in Bangladesh but has not been studied previously.

In a preliminary survey, larvae of cucumber moth, *Diaphania indica* (Crambidae: Lepidoptera) were found feeding on leaves of cucurbitaceous vegetables (Barmon *et al*., 2016). This pest is worldwide distributed and reported as a damaging pest in India (Radhakrishnan and Natarajan, 2009; Singh and Naik, 2006; Jhala *et al*., 2005; Chintha *et al*., 2002), Iran (Hosseinzade *et al*., 2014) Pakistan (Ashfaq *et al*., 2017), Sri Lanka (Ganehiarachchi, 1997), Japan (Kinjo and Arakaki, 2002; Inoue *et al*., 1982), China (Ke *et al*., 1986), Africa (Patel and Kulkarny, 1956), Australia (QFAB, 2012) and the USA (Capinera, 2001). It is a polyphagous insect (Viraktamath *et al*., 2003) and mostly prefers the Cucurbitaceous vegetables (Singh and Naik, 2006; Jhala *et al*., 2005; Viraktamath *et al*., 2003; Chintha *et al*., 2002; George *et al*., 2002; Inoue *et al*., 1982; Pandey, 1977). They are also reported as a harmful pest of non-cucurbitaceous plants such as plants under Leguminosae and Malvaceae families (Wakamura *et al*., 1998; Inoue *et al*., 1982).

Usually, one *D. indica* female lays around 250 eggs (Ganehiarachchi, 1997) but depending on the season they may lay more than 750 eggs (Ke *et al*., 1986). Eggs are small (0.33 mm × 0.83 mm), thin-walled and whitish (Ganehiarachchi, 1997). Females lay eggs preferably in clusters on the abaxial surfaces of leaves and occasionally on the other parts of their host plants (Ganehiarachchi, 1997). The young larvae cluster around the main veins, folding or binding leaves together and feed on the leaf epidermis (Barma and Jha, 2014; Patel and Kulkarny, 1956), while the older larvae are the voracious leaf feeder (Patel and Kulkarny, 1956). The larvae are likely to cause significant damage to the leaves and fruit productions if they are not treated properly (Jhala *et al*., 2005).

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The growth and development of *D. indica* are influenced by the variation of temperature (Halder *et al*., 2017; Barma and Jha, 2014) and relative humidity (Halder *et al*., 2017). Hence, the incidence and abundance of *D. indica* are likely to be different in different seasons in Bangladesh as the weather conditions in Bangladesh vary a lot in different seasons (Shahid, 2010). For instance, temperature varies from 23.9 ºC to 31.1ºC in the summer season and 7.2 ºC to 12.8ºC in the winter season in Bangladesh (Shahid, 2010). Attempts were made to find out the appropriate control measures of *D. indica* (Barmon *et al*., 2021) but understanding the host specificity, seasonal abundance and damage potentiality of *D. indica* would provide a comprehensive platform for developing sustainable management strategies. The current experiment was designed to study biology (life cycle), seasonal abundance and host choice of *D. indica*. Simultaneously, the impact of the weather parameters (temperature and relative humidity) on the abundance and damage potentiality of *D. indica* were studied to correlate their abundance in different seasons.

Material and Methods

A series of experiments were carried out in the field and in the laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh to study the field abundance, host preference, extent of damage, seasonal abundance of *D. indica* and the influence of local temperature and relative humidity on them.

Raising of plants. Three summer cucurbitaceous vegetables namely, Bitter gourd (variety: BARI Karola-1), Ridge gourd (variety: BARI Jhinga-1) and Snack gourd (variety: Local Chichinga) and three winter cucurbitaceous vegetablesnamely, bottle gourd (variety: BARI Lao 1), Cucumber (*Cucumis sativus* L.) and Squash (*Cucurbita pepo*) plants were used as host plants in the host preference tests. Quality seeds of selected vegetables were collected from the commercial seed dealers in Mymensingh town. Plants were grown following all recommended procedures including land preparation, application of fertilizers, intercultural operations.Initially, three seedlings were grown in every plot and only a single healthy seedling was kept per plot. Plants were then supported with a horizontal trellis (1.5 m × 1.5 m), made of bamboo for climbing. Plants were observed regularly and any pest insect on the plants was killed until 7 days before the starting of data collection.

Life cycle studies of *D. indica.* The life cycle of *D. indica* was studied in the laboratory at 25 ± 5ºC and 60 ± 5% RH. To have freshly laid eggs, one pair of adult moths (young and freshly emerged male and female) were kept in Petri dishes (10 cm in diameter) for mating and pieces of snake gourd leaves were provided as laying substrates in each Petri dish. At the bottom of the Petri dishes, moist paper towels were placed to keep the leaf turgidity. After mating, the male insect was removed and the female was allowed to lay eggs on the egg substrate. Five Petri dishes were set for the life cycle studies and the eggs laid on the egg substrates were observed daily to record the egg incubation periods, larval and pupal duration and adult longevity.

The layout of the host test experiments. Field experiments were conducted in two consecutive seasons (summer and winter) at the field laboratory of the Department of Entomology, Bangladesh Agricultural University. Experimental plots were laid out in a randomized complete block design (RCBD) method and each treatment was replicated three times. The experimental field (6 m × 6 m) was divided into 3 equal blocks of (2 m × 6 m) and the blocks were divided into 3 plots to have a total of 9 equal plots (2 m × 2 m). Spaces (≈ 25 cm) were kept in between adjacent plots for facilitating intercultural operations and experimental activities.

Host choice and seasonal abundance of *D. indica* in the field. Data recording on leaf infestations and larval abundance on plants were started when the plants were 35 days old and continued for four consecutive weeks at 7 days intervals. During the data recordings, the number of infested (by *D. indica*) and healthy leaves and the number of *D. indica* larvae per plant were recorded. The % leaf infestations and the number of larvae per plant were used in statistical analysis to interpret their host preference and abundance on summer and winter vegetables. Furthermore, the correlations of leaf infestations and the larval abundance with weather parameters were evaluated by comparing the recorded data with the fluctuations of temperature and relative humidity during the trial. Data on temperature and relative humidity of respective seasons were collected from the Department of Irrigation and Water Management, Bangladesh Agricultural University, Mymensingh. In general, the summer season is hot and cloudy but the winter season is cool and clear in Mymensingh (Akhter *et al*., 2019). The mean annual temperature varies from 13 to 33 ºC and sometimes goes below 10 ºC and above 37 ºC (Akhter *et al*., 2019).

Statistical analysis. Normality of data was evaluated by Shapiro-Wilk tests and by examining visually the distribution of the residuals of models. When data were not normally distributed, log-transformed data [$\log_{10}(x)$] were used in the analysis and the original data (not transformed) were used for the visualisation. Two-way ANOVAs of the linear models (for % leaf infestations) and GLM models (considering the poisson distribution for the data of larval abundance per plant) were performed followed by fisher's least signifcant difference (LSD) tests to differentiate the levels of predictors. To know the difference between leaf infestation in summer and winter vegetables and larval abundances in summer and winter vegetables, independent t-tests were performed. Correlation and regression analyses were done to find out the relationships between larval abundance and weather parameters (temperature and relative humidity) during the trial. Statistical analyses were done using the statistical program "R" (R Core Team, 2017) and Microsoft Excel 2016 (Microsoft Corporation, 2016).

Results and Discussion

Life cycle studies of *D. indica*. The duration of the complete life cycle (from eggs to adults) of *D. indica* was 17.4 ± 0.36 days (Fig. 1). Eggs were laid in clusters and usually covered with scales. Eggs were hatched into larvae in 3.5 ± 0.16 days. Larvae were green with two pale lines along their back. Larvae rolled the leaves with the help of white and silky threads and fed inside. The total larval duration was 12.4 ± 0.16 days. There were 5 larval instars, and the duration of 1st, 2nd, 3rd, 4th and 5th larval instars were 2.8 ± 0.14, 2.6 ± 0.17, 2.4 ± 0.17, 2.4 ± 0.17 and 2.2 ± 0.14 days respectively. Within the rolled leaves, 5th instar larvae transformed into pupae. The pupal duration was 5 ± 0.22 days before adult eclosion. The wings of the adults were white and transparent with distinct brown margins. At the end of the abdomen, a bunch of brown hairs were present. The adult longevity was 7.4 ± 0.28 days which was not significantly different for males and females.

Host choice and seasonal abundance of *D. indica* in the field. Percent leaf infestations were varied for

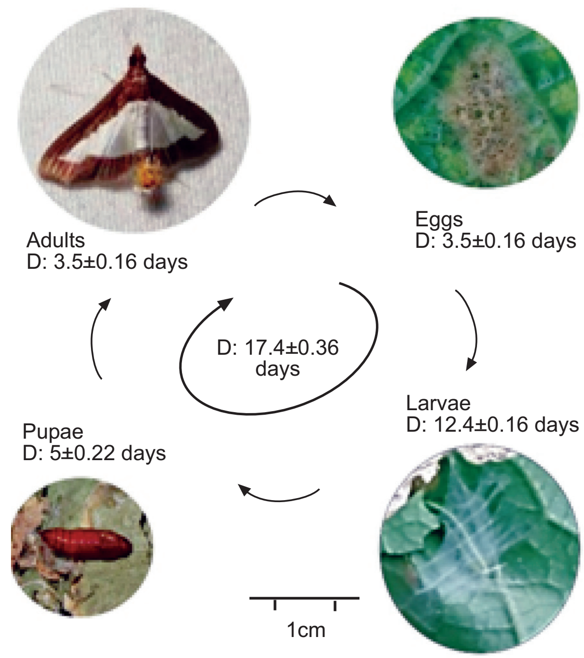

Fig. 1. The life cycle of *D. indica*; durations/ longevity (D) in days (± SE) of life stages.

summer vegetables (2 Way ANOVA: $F_{2,24}$ = 9.51; P<0.001) and for different data recordings (2 Way ANOVA: $F_{3,24}$ = 43.01; P<0.001) (Fig. 2). Maximum leaf infestations were found on snake gourd which was significantly higher than the leaf infestations on the bitter gourd and ridged gourd plants (LSD; P<0.05). However, % leaf infestations were not different between the bitter gourd and ridged gourd plants (LSD; P>0.05). Increased leaf infestations were evident in the latter data recordings than the earlier data recordings. At first data recording, over 15% leaf infestations were recorded which became more than 30% at the 4th data recording. At the 4th data recordings, maximum leaf infestations were recorded (LSD; P<0.05). However, % leaf infestations in the first 3 data recordings were not different (LSD; P>0.05). On the other hand, very minimum leaf infestations (< 5%) were found in the winter season which was significantly lower than the % leaf infestations on summer vegetables (t= 23.01, df= 53; P<0.001). The % leaf infestations in the winter season were not statistically different for vegetables (2 Way ANOVA: $F_{2,24}$=0.41; P>0.05) and different data recordings (2 Way ANOVA: $F_{3,24}$=0.67; P>0.05).

Similar to the % leaf infestations, larval abundance per plant varied significantly for vegetable varieties (2 Way

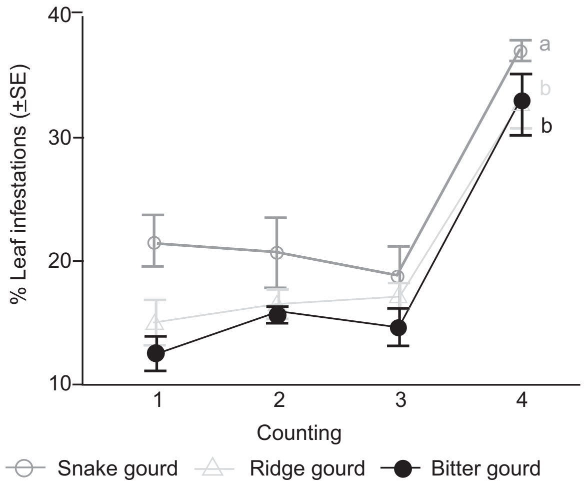

Fig. 2. Percent leaf infestations (± SE) of summer vegetables by *D. indica* (2 Way ANOVA: $F_{2.24} = 9.51$; $P<0.001$) over successive data recordings (2 Way ANOVA: $F_{3.24} = 43.01$; $P<0.001$). No interactions were found between the data recordings and vegetables (2 Way ANOVA: $F_{6.24} = 1.01$; $P>0.05$). Vegetables marked with the same letters are not different (LSD test; $P>0.05$).

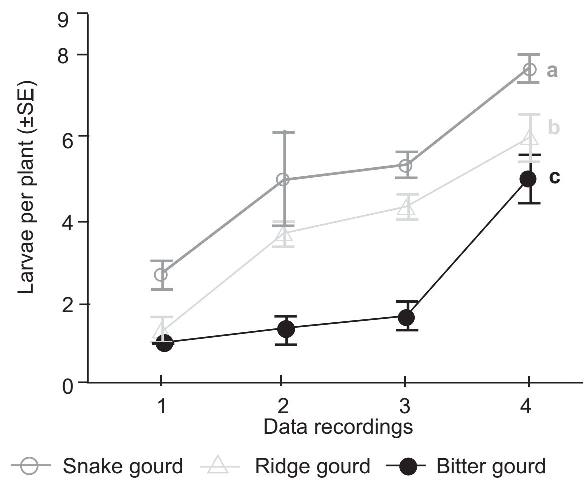

Fig. 3. Mean number (± SE) of *D. indica* larvae per plant (2 Way ANOVA: $F_{2.30} = 7.09$; $P<0.001$) over successive data recordings (2 Way ANOVA: $F_{3.32} = 8.61$; $P<0.001$). No interactions were found between the data recordings and vegetables (2 Way ANOVA: $F_{6.24} = 0.73$; $P>0.05$). Vegetables marked with the same letters are not different (LSD test; $P>0.05$).

ANOVA: $F_{2,30} = 7.09$; $P<0.001$) and the date of the data recordings (2 Way ANOVA: $F_{3,32} = 8.61$; $P<0.001$) in the summer season (Fig. 3). The maximum larval abundance was found on snake gourd plants (LSD; $P<0.05$) and the minimum larval density was found on bitter gourd plants (LSD; $P<0.05$). The larval abundance on ridge gourd was significantly higher than on bitter gourd (LSD; $P<0.05$) and lower than on snake gourd (LSD; $P<0.05$). During the winter season, very few larvae (mean number =1) were observed on winter vegetables. On the winter vegetables, larval presence was not statistically different considering the vegetable types (2 Way ANOVA: $F_{2,30}= 0.69$; $P<0.05$) and the advancement of growing season (2 Way ANOVA: $F_{2,32}= 0.67$; $P<0.05$). Overall, the larval presence was significantly lower on winter vegetables than on summer vegetables ($t= 9.10$, $df= 39$; $P<0.001$).

Effects of weather parameters on the abundance of *D. indica.* The relationships between larval abundance on the summer vegetables and the temperature fluctuations were presented in Fig. 4. The larval abundance was increased on the bitter gourd, ridge gourd and snake gourd with the increase of temperature. The significant positive correlations were found for bitter gourd ($r = 0.63$; $P<0.01$) and snake gourd ($r = 0.95$; $P<0.01$) but for the ridge gourd, the relationship was positive but not significant ($r = 0.63$; $P>0.05$).

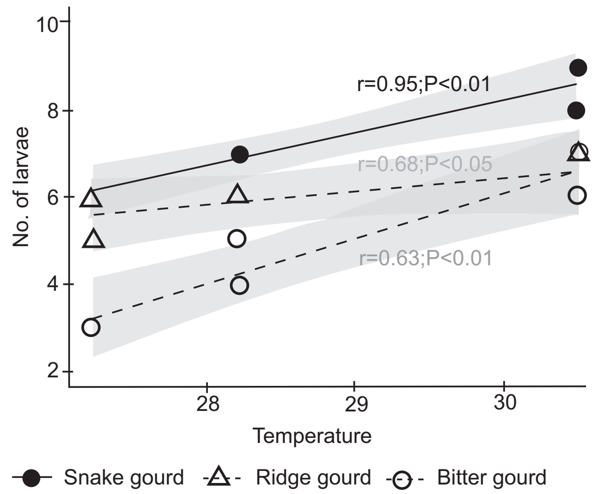

Fig. 4. Relationship between the temperature and the larval abundance on snake gourd, ridge gourd and bitter gourd during the summer season. The grey shaded areas represent the ± SE of the respective lines.

On the contrary, negative correlations between larval abundance and % relative humidity on summer vegetables were found during the trial (Fig. 5). Increased larval abundances on the bitter gourd, ridge gourd and snake gourd were found for the reduced % relative humidity. Strong negative relationships between % relative humidity and larval abundance was found for bitter gourd (r = -0.87; P<0.05) and snake gourd (r= -0.85; P<0.05) and for the ridge gourd, the relationship was not significant (r= -0.65; P>0.05).

During the summer season, *D. indica* was likely to be a significant damaging pest of cucurbitaceous vegetables (Fig. 2-3). The % leaf infestations (up to 35%) and larval abundance (up to 8 larvae per plant) were higher on the selected summer vegetables than the selected winter vegetables (Fig. 3). Possibly, the higher temperature in the summer season provided favourable conditions for the development and establishment of *D. indica*. The regression analysis between the temperature and larval abundance showed the larval abundance was positively influenced by the temperature (Fig. 4). The warm weather condition in the summer season in Bangladesh (Shahid, 2010) provided favourable environmental conditions for building increased larval abundance per plant. Previous studies (Choi *et al*., 2003; Peter and David, 1991) also confirm that the summer season is the favourable season for *D. indica*. As in the winter season, a very minimum level of leaf infestations

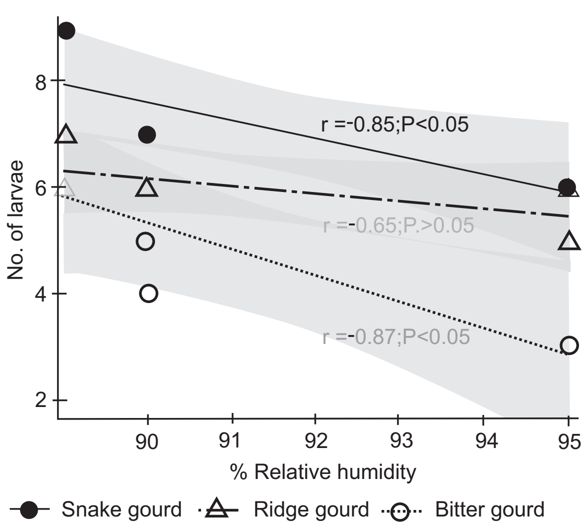

Fig. 5. Figure relationship between relative humidity (%) and larval abundance on snake gourd, ridge gourd and bitter gourd during the summer season. The grey shaded areas represent the ± SE of the respective lines.

and larval abundance were found, the data on larval abundance on winter vegetables were excluded from the correlation regression studies between weather parameters and larval abundance.

Insects are poikilothermic, their metabolic rates, growth and development vary with the variation of weather parameters (Johnson *et al*., 2016; Li *et al*., 2013). The growth and development of insects depend on the temperature (Khaliq *et al*., 2014; Regniere *et al*., 2012). The increased temperature speeds up the physiological process of insects (Regniere *et al*., 2012; Rajasekhar *et al*., 2005) and reduces the life cycle durations. As a result, a higher number of generations are produced in a season. Furthermore, *D. indica* are highly fecund (Ganehiarachchi, 1997; Ke *et al*., 1986) and completed their life cycle in less than a month (<20 days) (Fig. 1). As a result, they may build up a bigger population in their favourable conditions. The results of the current studies also support the aforesaid facts that the *D. indica* population gets bigger with the age of the plants (Fig. 3). With the advancement of the cropping season, *D. indica* increases its number and may cause more harm to the plants. Most of the cucurbitaceous vegetables in Bangladesh are summer vegetables (Ali and Hau, 2001). During the summer season, these vegetables become the principal vegetables in the vegetable markets (Ali and Hau, 2001). Therefore, any damage or threat to the summer cucurbitaceous vegetables is a big concern for the supply of quality vegetables in the summer season.

Similar to the temperature, insects have a favourable range of relative humidity (Khaliq *et al*., 2014). Above or below that range, their normal physiological growth and development are hampered. The larval abundances were found negatively correlated to the relative humidity (Fig. 5). The increased relative humidity affected the larval abundance (Halder *et al*., 2017; Norhisham *et al*., 2013; Singh and Naik, 2006; Rajasekhar *et al*., 2005).

Among the summer vegetables, the bitter gourd was less susceptible and the snake gourd was the most susceptible to *D. indica* considering both the % leaf infestations (Fig. 2) and larval abundance (Fig. 3). In previous studies (Kamal *et al*., 2014; Kamal *et al*., 2013), the bitter gourd has been proven as resistant to some other major pests of cucurbitaceous vegetables. However, any damage to the leaves may result in a qualitative and quantitative loss in ultimate production. *Diaphania indica* larvae rolled leaves with a help of silken threads which compromises the active photosynthetic areas of leaves. Bitter gourd leaves are smaller than snake gourd and ridge gourd leaves. Fewer larvae on bitter gourd plants may likely contribute similar impacts as impacts of the higher number of larvae on snake gourd and ridge gourd leaves. Moreover, the excreta of *D. indica* left on the damaged leaves may attract fungus to cause secondary fungal infections (observed during conducting the experiments). Therefore, similar to other major pests of cucurbitaceous vegetables, *D. indica* is to be considered as a target pest and appropriate control measures should be taken to keep *D. indica* under a certain limit. However, the impact assessment and the distribution studies covering all of the representative cucurbitaceous vegetable cultivation areas of Bangladesh are recommended to compare their damage with the existing major pests of cucurbitaceous vegetables.

Conclusion

Larval abundance and leaf infestations were higher in the summer season than in the winter season. Among the selected summer vegetables, the bitter gourd was the least preferred, the snake gourd was the most preferred and the ridge gourd was moderately preferred host to the *D. indica*. Larval abundance was affected by the variation of temperature and relative humidity. The temperature was found to be influential in increasing larval abundance. On the other hand, relative humidity played the opposite role of temperature and negatively influenced larval abundance. The life cycle duration of *D. indica* was 17.4 ± 0.36 days where, the incubation period, larval period and adult longevity were 3.5 ± 0.16, 12.4 ± 0.16 and 3.5 ± 0.16 days respectively. Leaf infestations and larval abundances were increased with the advancement of the cropping season. Therefore, proper control measures are recommended to reduce the possible crop loss by them.

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Conflict of Interest. The authors declare they have no conflict of interest.

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