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Population Fluctuation of Rice Leaffolder, (*Cnaphalocrocis medinalis*) in Two Consecutive Rice Seasons

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ABSTRACT

Larvae of rice leaffolder, *Cnaphalocrocis medinalis* attack rice crop at all three phases (from tillering until maturity stages). Their population throughout season was influenced by abiotic and biotic factors. Study on population fluctuation is important to understand the population dynamic and its factors in natural field condition. Thus, a study of population fluctuation of *C. medinalis* was conducted at rice field in Semanggol, Perak. Larvae were collected from 10 rice hills per plot in three 15 m x 4 m plots started on April 5, 2015 until June 7, 2015 (off season) and from October 25, 2015 until December 27, 2015 (main season). Abiotic data namely temperature, relative humidity (RH) and rainfall were obtained from Department of Meteorology Malaysia. Comparison of *C. medinalis* population between two seasons at different weeks was analysed using two-way ANOVA. The relationship of *C. medinalis* population with abiotic factors was analysed using Pearson correlation and stepwise regression. There was a significant interaction effect between season and week ($F_{9,40}=2.19$; $p<0.05$). The highest population was recorded at week 55 day after transplanting (DAT) in main season comprised of 17% of total collection, followed by week 62 in off season (14%), week 48-main season (13%) and week 55-off season (13%). There was a positive correlation between *C. medinalis* population and RH in main and off season with RH was the key factor in regulating population in both seasons. Our results revealed that population of *C. medinalis* at different weeks was influenced by season. It is due to differences of weather condition between seasons, management practices and plant stages. The highest population recorded during reproductive phase is due to the morphology of rice plant that offers great suitability for larvae feeding and development. The abiotic factors also influenced *C. medinalis* population. Therefore, the abiotic factors should be considered in management of *C. medinalis* besides the plant stage and fertilizer effect. Future research on effect of natural enemies on *C. medinalis* population in field is needed in order to gain a better understanding of the factors that influence the population.

Keywords: Abiotic factor, *Cnaphalocrocis medinalis*, population fluctuation, rice pest, rice stages

INTRODUCTION

Rice leaffolder, *Cnaphalocrocis medinalis* is an insect rice pest belongs to family Pyralidae and genus *Cnaphalocrocis*. The larvae attack rice crop by folding rice leaves and scrape the green mesophyll tissue (chlorophyll) from within (Punithavalli et al., 2011). The larvae attack rice crop at all three phases starting from tillering until maturity stage (Patel et al., 2011). Their occurrence was recorded in every rice crop season (Bhumireddy et al., 2018).

Their population throughout the season is influenced by many factors comprising of abiotic and biotic factors. Abiotic factors such as temperature, relative humidity, rainfall and the management practices relate to the changes in abundance, distribution and population dynamics of the insects (Ayres and Schneider, 2009; Mondal and Mandal, 2017). The biotic factors including host plant and parasitoid are responsible for the fluctuation of the population (Karl et al., 2011; Salmah et al., 2019). Population of *C. medinalis* fluctuated with different rice plant stages (Islam and Karim, 1997). The larvae population and their damage was significantly different among rice varieties (Punithavalli et al., 2013).

Study on population fluctuation is important to understand the population dynamic of the organism and its factors in natural field condition (Pasinelli et al., 2011). Understanding the population dynamic of insect pests plays vital role in integrated pest management strategy (Dutta and Roy, 2018). Therefore, many studies on population fluctuation of *C. medinalis* were conducted by previous researchers in understanding the population dynamics (de Kraker et al., 1999; Alvi et al, 2003; Chakraborty and Deb, 2011; Ram et al., 2014; Kakde and Patel, 2015, Nirala et al., 2015; Sulagitti et al., 2017; Zainab et al., 2017; Bhumireddy et al., 2018). The results of these studies are not exactly similar to each other due to different factors that influenced the population which depends on the field condition of the studied area. Thus, different rice fields may have different pattern of population fluctuation which are influenced by different factors.

To date, there is no published data on population fluctuation of *C. medinalis* in Malaysian rice fields. Although there has been no record of its outbreak in Malaysia for the last 20 years, the risk of the outbreak is possible. This is due to the fact that its existence in local paddy fields has been reported in several studies (Chang, 1992; KADA, 2011; Marina et al., 2019). Moreover, *C. medinalis* has history of outbreak in Sekinchan, Selangor as reported by Ooi and Yazid (1982). It was considered as minor pest before evolving as major pest in late 1980's in many parts of the world including Malaysia due to increase in abundance (Hafeez et al., 2010). The evolvement was influenced by the agricultural practices used by farmers, climatic conditions and pest management regimes (Bergé and Ricroch, 2010). Thus, this study aimed to compare the population fluctuation of *C. medinalis* between two consecutive seasons and between different weeks, and to determine the relationship of *C. medinalis* population with the abiotic factors.

MATERIALS AND METHODS

Study area

The study was conducted at rice field located in Kampung Jalan Gula, Semanggol, Perak, Malaysia (4° 57. 0300' N, 100° 36.7333' E). It is located in one of granary areas known as Integrated Agriculture Development Area (IADA) Kerian - Sungai Manik. Field was planted with local high yield rice variety, MR220 CL2 that have early maturation period of 91 days according to FGG (2016). Three plots were set up in 100 m distance from each other with at least 5 m distance from bund. The area of the sampling field is about 2.0 acre. Management practices of this field was the common management practices used by most farmers in Malaysia which involve application of chemical pesticides and fertilizers. The insecticides used in the field were Alika ZC (0.195 L ha^{-1}) and Karate zeon (0.100 L ha^{-1}) while fertilizers were NPK compound (contain nitrogen, phosphorus and potassium) and urea fertilizers (contain nitrogen). Farmer has applied insecticides twice from the beginning of season until the last date of sampling. Insecticide with lambda cyhalothrin as active ingredient was used to control insects during vegetative phase and insecticide with thiamethoxam and lambda cyhalothrin as active ingredient was applied during reproductive phase. Fertilizers were applied three times throughout the season at

114:43:54 kgha^{-1}. This rice field was surrounded by other rice fields, near to farmers' houses and with proper irrigation system.

Sampling method

Sampling of larvae was carried out by visual searching on folded leaves and collected by hand-picking from 10 rice hills per plot in three 15 m x 4 m plots. Selection of 10 hills was done by randomly selecting two hills from five 4 m x 3 m subplots in each plot. Sampling was carried out in weekly interval in two consecutive seasons namely main and off season. Main season is known as wet season with high rainfall amount during the season and paddy planting does not depend wholly on irrigation system. In contrast, off season is known as dry season as the location receive low rainfall during the time interval and paddy planting normally depends on irrigation system (DOA, 2016). Sampling started on April 5, 2015 (13 day after transplanting, DAT) until June 7, 2015 (76 DAT) in off season and from October 25, 2015 until December 27, 2015 in main season, from 0800 – 1100 hours due to active time for diurnal insect in foraging activity and suitable time with adequate light intensity to find larvae in the field. Collected larvae were put in plastic container with diameter of 7.5 cm and height of 7.5 cm that has been punched using insect pin to allow air ventilation. The container was labelled corresponding to plot number. The collected samples were brought to laboratory for enumeration process. The sampling method was adopted from Alvi et al. (2003).

Data of three abiotic factors namely temperature, rainfall and relative humidity were obtained from Department of Meteorology Malaysia that were recorded at Chersonese Estate Kuala Kurau Station (5° 00' N, 100° 26' E). Data of seven days toward sampling date were pooled to get mean values.

Samples enumeration process

The number of *C. medinalis* larvae collected from 10 rice hills in each plot were recorded. The number of individuals collected was divided by 10 to obtain the mean number of larvae per hill as sampling unit. Similar process was done for each sampling date in both seasons.

Data analysis

All data was tested for normality using normality test. The abnormal data was normalized using $\log_{10}(x+1)$ transformation. Comparison of *C. medinalis* population (number of larvae per hill) between two seasons and at different weeks was analysed using two-way ANOVA and multiple means comparison using Fisher's Least Significant Difference (LSD) method. The comparison of *C. medinalis* population (number of larvae per hill) at each week between two seasons was analysed using two sample t-test. The relationship of *C. medinalis* population with abiotic factors was analysed using Pearson correlation and stepwise regression. All analyses with significance level at $P = 0.05$. All analyses were performed using software Minitab version 16.0.

RESULTS AND DISCUSSION

Comparison of population fluctuation of *C. medinalis* between two seasons and between different weeks

There was a significant effect of interaction between season and week ($F_{9,40} = 2.19$; $p < 0.05$) (Fig. 1). The highest population was recorded at week 55 DAT in main season (0.50 ± 0.06) and followed by week 62 in off season (0.43 ± 0.15), week 48-main season (0.40 ± 0.06) and week 55-off season (0.40 ± 0.06). The population at the four weeks was significantly different with other weeks except week 62 DAT in main season (0.33 ± 0.09). The lowest population was recorded at week 20-main season (0.03 ± 0.03) and there was no larva found at week 13 and 76 in main season and at week 13, 69 and 76 DAT in off season.

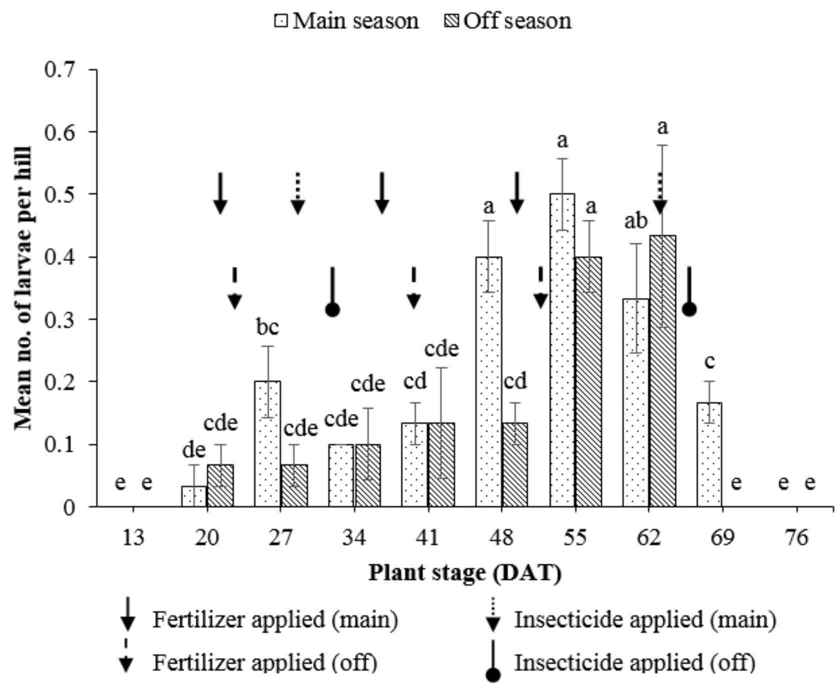

Fig. 1. Population of *C. medinalis* in main and off season at different weeks corresponding to the date of fertilizer and insecticide application. Means with the same letters are not significantly different (P>0.05) by LSD method. The error bars indicate standard error.

Our results revealed that *C. medinalis* population at different weeks depends on rice season. It is due to difference of weather condition between the two seasons. Besides that, management practices throughout the season and different plant stages have influenced the population at different weeks. It was found that higher population was recorded in wet season (main season; August to November) while lower population in dry season (off season; February to May) (de Kraker et al., 1999; Padmavathi et al., 2015). The injured leaves were higher in wet season compared to dry season (de Kraker et al., 1999). Leaf injury per crop was positively correlated with larval infestation level. Furthermore, *C. medinalis* completed more generations which were three during wet season whilst two generations during dry season (Padmavathi et al., 2015).

During main season, farmer applied fertilizer during 22, 39 and 50 DAT, respectively while insecticide was applied during 29 DAT and 63 DAT. In off season, fertilizer was applied on 22, 40 and 50 DAT, respectively while insecticide was applied on 30 and 64 DAT. It was observed that population gradually increased after fertilizer was applied (Fig. 1). It could be related to the increase of nitrogen content in rice leaves as compound and NPK fertilizers are composed of three macronutrients namely nitrogen (N), phosphorus (P) and potassium (K). Nitrogen is the most essential nutrient needed by plant for growth and development. Thus in host plant, the food quality for foliage feeder is indicated by nitrogen content in leaves. Plants that have absorbed nitrogen will produce green and healthy leaves (Lu et al., 2007). The green leaves reflect the green mesophyll cell or pigment in leaves. The palisade parenchyma cells of the mesophyll plays important function in food production process called photosynthesis (Yahia et al., 2019).

Therefore, green leaves attract rice leaffolder females to lay eggs and larvae prefer to feed on green leaves. There was a positive correlation between oviposition preference and host plant quality in ensuring their offspring development and survival (Liao and Chen, 2017). This is due to the immobility of their neonate larvae (Shikano et al., 2010; Marina et al., 2019). Chemical emanation from surface of plant tissues influenced the selection of suitable host plants by female adults for oviposition (Udayagiri and Mason, 1995). This is due to the significant effect of rice plant volatiles on *C. medinalis* behaviour by attracting the pests toward rice plant, stimulating their feeding and guiding them in choosing oviposition sites (Du, 2001; Liu et al., 2012). A study on olfactory response of *C. medinalis* towards various volatile compounds of plant origin using electroantennograms (EAGs) revealed a strong EAG response displayed by moths towards green leaf odour (Ramachandran and Khan, 1991). This could explain the significant higher oviposition preference of female *C. medinalis* for resistant and susceptible cultivars compared to resistant wild rice (Liao and Chen, 2017). High level of nitrogen fertilizer treatment demonstrated more laid eggs, higher larval survival rate and percentage of injured leaves compared to the treatment with low level nitrogen fertilizer (de Kraker et al., 2000). Nitrogen content also affected several bionomic characteristics of rice leaffolder such as the increase of leaf area consumption by larvae and their survival rate, pupal weight, the longevity, fecundity and preference of oviposition of adult (de Kraker et al., 2000).

Although high nitrogen content in rice leaves has positively affected the oviposition of adult *C. medinalis* however, the application of insecticides has contributed to the population decrease and remained low. This is due to the fact that chemical insecticide is known to be one of the pest management methods that successfully controlled many pests (Vastrad, 2011; Zheng et al., 2011). Thus, the fluctuation of leaffolder population was affected by application of insecticides. A study revealed the leaffolder mortality ranged from 76 to 100% after 24 hours applied (Alvi et al., 2003). The reduction in *C. medinalis* population ranged from 44 to 65% on 1 day after spray (DAS), 47 to 70% on 3 DAS and 41 to 76% on 7 DAS (Khuhro et al., 2014). Due to fast effect and distinct result, most farmers prefer to apply chemical insecticides as it has been proven that treated field produced higher yield compared to untreated field (Alvi et al., 2003; Khuhro et al., 2014). However, the continuous use of insecticide can lead to pest resistance problem (Kaushik, 2010).

Population trend of *C. medinalis* in two different rice seasons throughout the season showed colonization started during second week of sampling which was in vegetative phase (Fig. 2). Then, it fluctuated until ninth week in main season and until eight week in off season (ripening phase). The growth phase of rice plant was differentiated based on the observation on plant morphology. Vegetative phase started from early season until maximum tillering and panicle initiation stage which also the early of reproductive phase (Moldenhauer et al., 2013). However, the characteristic of maximum tillering and panicle initiation stage of rice plant was difficult to be recognized by naked eye. Thus, the early reproductive phase was estimated based on flowering date which is the end of reproductive phase and also early ripening phase (Moldenhauer et al., 2013). The reproductive phase lasts about 35 days in most varieties (IRRI, 2015). Hence, the early of reproductive phase was estimated by subtracting the date of flowering by 35.

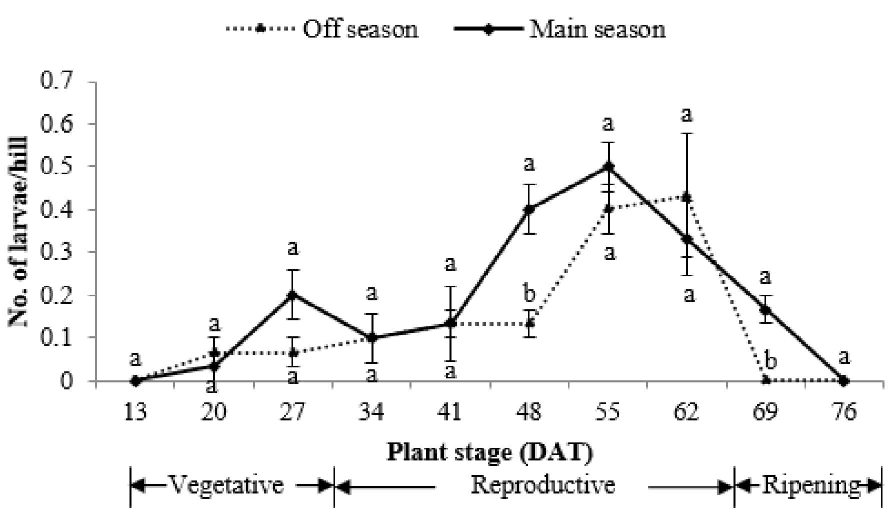

Fig. 2. Population trend of *C. medinalis* in two different rice seasons. Means with the same letters at the same week are not significantly different (P>0.05) by LSD method. The error bars indicate standard error.

The population in main season was low at second week (20 DAT) and increased during third week (27 DAT) before decreased during fourth week (34 DAT) and continue increased afterward with sharply increased during sixth week (48 DAT) with highest larvae collected during seventh week (55 DAT). Then, the population started to decrease during eight week (62 DAT) and continued until tenth week (76 DAT) with no larva collected. In off season, the population has gradually increased during first six weeks (13 until 48 DAT) and sharply increased during seventh week and continued increase until peak during eight week. During ninth week, the population has sharply decreased with no larvae was found which similar during tenth week. There were two weeks that population between two seasons differed significantly. It was sixth ($T = 4.17$; df = 1, 4; $P < 0.05$) and ninth week ($T = 5.27$; df = 1, 4; $P < 0.05$).

In general, the pattern of population fluctuation in two seasons has resemblance with each other. Population was low at the beginning of season (vegetative phase) and gradually increase until the peak during reproductive phase before gradually decreased during ripening phase towards the end of season. This result is in close proximity to de Kraker et al. (1999), Alvi et al. (2003), Ram et al. (2014), Kakde and Patel (2015), and Zainab et al. (2017) who observed the similar pattern of fluctuation. However, the recorded number of larvae per hill in their studies was higher than this study. It can be related to free insecticide field in their experiment. Thus, the fluctuation of *C. medinalis* population in this study was contributed by application of insecticide and fertilizer besides plant stage.

de Kraker et al. (1999) found the colonization of rice leaffolder larvae in rice field was related to oviposition by the adults. They observed moths usually first appeared in four weeks after transplanting with eggs peaked around maximum tillering stage (7 weeks after transplanting) and larvae population was at peak after one or two weeks later at booting stage (reproductive phase). Therefore, it can be concluded that low abundance of *C. medinalis* larvae during vegetative phase in this study is related to the low abundance of *C. medinalis* moths with low oviposition during the early season. The oviposition preference is probably related to the quality of the host plant (Liao and Chen, 2017; Marina et al., 2019). Rice plant during early vegetative phase is young with simple root system, narrow and less number of leaves (Dunand and Saichuk, 2009). Hence, the plant is less efficient in absorbing nutrient especially nitrogen which resulted in less content of nitrogen in leaves. The less content of nitrogen in leaves contribute to the less oviposition by female moths during early vegetative phase. Furthermore, the leaves are less suitable for leaffolder larvae which is the foliage feeder as they need green, healthy and wider rice leaves (Islam and Karim, 1997; Lu et al., 2007).

Meanwhile, the lower population was recorded during ripening phase is due to the physical properties of the rice plant which was unsuitable for development and survival of leaffolder larvae. The leaves are narrower, tougher and older (Heong, 1990; Punithavalli et al., 2011). Narrower and tougher leaf blades were often rejected by larvae (Islam and Karim, 1997). Hence, the morphology of rice leaf blade including leaf blade width and length, and toughness play an important role in resistance against *C. medinalis* (Punithavalli et al., 2011). It was suggested that the low larvae population during ripening phase was due to the reason that no more new leaves were produced (Ram et al., 2014). In addition, only few eggs were found after the flowering stage (de Kraker et al., 1999). In a choice situation, *C. medinalis* moths laid most eggs in the tillering crop but in a no-choice situation, *C. medinalis* moths that emerged after flowering stage did not oviposit in the same crop but they emigrated (de Kraker et al., 1999). The similar finding was reported as there were numerous adults were found at the pre-harvest stage but egg laying was not observed either in the rice fields or in the surrounding alternative hosts (Padmavathi et al., 2015). It was concluded that the unavailability of suitable stage of the rice plant in fields and lack of adult nutrition are the main causes for the absence of eggs on host plants in spite of numerous adults present at pre-harvest stage of the crop.

On the other hand, the high population of *C. medinalis* larvae during reproductive phase can be related to the morphology of rice plant during that phase. It offers great suitability for larvae feeding. The rice plant is composed of fully developed main shoot and healthy green leaves during reproductive phase (Dunand and Saichuk, 2009). The leaves grow and become wider and longer during this period. Punithavalli et al. (2011) revealed that leaves selection by larvae was based on morphology of rice leaf blade. Larvae of leaffolder preferred wider, softer and flexible leaves to fold and feed (Islam and Karim, 1997; Punithavalli et al., 2011). Moreover, the high oviposition around maximum tillering (end of vegetative phase) stage which is the most preferred by rice leaffolders for oviposition (de Kraker et al., 1999) also contributed to the high population of *C. medinalis* larvae during reproductive phase. Besides that, the maximum adult percent in peak egg deposition stages was found at the panicle initiation stage (early reproductive phase) (Padmavathi et al., 2015).

Determination of relationship of *C. medinalis* population with abiotic factors

Table 1 shows the relationship of *C. medinalis* population with abiotic factors namely relative humidity, temperature and rainfall. From the result, it shows a significant positive correlation between abundance of *C. medinalis* and relative humidity in both main (r = 0.64) and off season (r = 0.72). There was no significant correlation for temperature and rainfall towards the abundance of *C. medinalis* in both seasons. The result indicates that abundance of *C. medinalis* increased with increasing of relative humidity. The positively influence of relative humidity towards *C. medinalis* population in both seasons was similar to previous studies (Chakraborty and Deb, 2011; Kakde and Patel, 2015; Nirala et al., 2015; Sulagitti et al., 2017; Zainab et al., 2017).

Table 1. Correlation of *C. medinalis* population against abiotic factors

Season	Predictor variable	r	P value
Main	Relative humidity	**0.64**	< 0.05
	Temperature	0.38	> 0.05
	Rainfall	0.05	> 0.05
Off	Relative humidity	**0.72**	< 0.05
	Temperature	0.31	> 0.05
	Rainfall	0.26	> 0.05

Further relationship between *C. medinalis* population and abiotic factors were described by stepwise regression (Table 2). The result shows that relative humidity was the key factor in regulating *C. medinalis* population in both main ($R^2 = 0.34$) and off ($R^2 = 0.43$) season. This result explained relative humidity has contributed 34% in variation of *C. medinalis* population in main season and 43% in off season. Temperature also affected the population of *C. medinalis* by 19% ($R^2 = 0.19$) although it was not significant. On the other hand, all abiotic factors have caused variation in *C. medinalis* population in off season. Temperature contributed 26% ($R^2 = 0.26$) while 13% ($R^2 = 0.13$) contribution from rainfall.

Table 2. Stepwise regression for *C. medinalis* against abiotic factors

Season	Predictor variable	R^2	P value
Main	Relative humidity	0.34	< 0.05
	Temperature	0.19	> 0.05
Off	Relative humidity	0.43	< 0.05
	Temperature	0.26	< 0.05
	Rainfall	0.13	< 0.05

From the results, it can be concluded that the three abiotic factors namely relative humidity, temperature and rainfall has contributed to the changes in population of *C. medinalis* with relative humidity contributed more than others. The positive influence of relative humidity, temperature and rainfall towards *C. medinalis* population was also reported in other studies. Nirala et al. (2015) and Zainab et al. (2017) found the significant and positive correlation of relative humidity and rainfall with *C. medinalis* population. A significant positively correlation of relative humidity and non-significant positively correlation of rainfall has been reported by Chakraborty and Deb (2011) while Kakde and Patel (2015) and Sulagitti et al. (2017) reported a positive correlation of relative humidity (significant), temperature (non-significant) and rainfall (non-significant).

Weather components such as relative humidity, temperature and rainfall have known to greatly influence the insect population directly by limiting or expanding their distribution, growth, reproduction, diapause and dispersal (Win et. al, 2011; Karuppaiah and Sujayanad, 2012; Supawan and Chongrattanameteekul, 2017). At the same time, the components indirectly influence through plant mechanisms and natural enemies that regulate the insect population (Heong et al., 2007; Siswanto et al., 2008). The components also can affect various insect immune and genetic responses (Khaliq et al., 2014).

Baskaran et al. (2017) stated that relative humidity was supportive to the multiplication of leaf-folder. High humidity support reproductive capacity in insects as percentage of water is correlated with the amount of fat including eggs in females and with the amount of cuticle (Norhisham et al., 2013). This is because the low relative humidity can prevent embryo development and egg hatching due to loss of lubrication and cuticular softness in insect (Guarneri et al., 2002). Dehydration occurred at low relative humidity due to loss of moisture in the egg, which lead to contraction and shrinking of both chorion and the embryo (Norhisham et al., 2013).

Meanwhile, Wan et al. (2015) concluded that there was significant effect which is in positive way of average daily temperature on the occurrence of *C. medinalis*. It is due to insects requirement for a certain amount of heat units (degree days) to develop from one life stage to the other (Padmavathi et al., 2013). It is due to their dependency on the temperature of the surrounding environment in changing their activity as insects is a poikilothermic animal (Bale et al., 2002; Menéndez, 2007). Increasing the temperature to the thermal optimum level causes acceleration of the insect metabolism (Jaworski and Hilszczański, 2013). Thus, Bhumireddy et al. (2018) concluded that the hot and humid environment wherein rice is grown is highly conducive for proliferation of insect pests resulting in serious outbreak.

However, there are some studies found non-significant correlation of all of weather factors towards *C. medinalis* population (Bhumireddy et al., 2018) while some reported a significant negative relation between the weather factors with per cent leaf damage of leaf folder (Khan and Ramamurthy, 2004; Patel et al., 2011). These variations might be due to variation in weather parameters in different locations and their influence on activity of the pest.

CONCLUSION

As a conclusion, the population of *C. medinalis* at different weeks was influenced by season. The population fluctuation in both main and off season has similar pattern as the population was low at the beginning of season (vegetative phase) and gradually increase until the peak during reproductive phase before gradually decreased during ripening phase towards the end of season. The highest population was recorded during week 48, 55 and 62 DAT which categorized into reproductive phase. It is due to morphology of rice plant during reproductive phase offers great suitability for larvae feeding and development. The population of *C. medinalis* had positive correlation with relative humidity while no correlation with temperature and rainfall. However, further analysis using stepwise regression showed the significant and positive relationship between *C. medinalis* population and the three abiotic factors (relative humidity, temperature and rainfall). It can be concluded that the abiotic factors has contributed to the changes in population of *C. medinalis*. Therefore, the abiotic factors should be considered in management of *C. medinalis* besides the plant stage and fertilizer effect. Furthermore, future research on effect of natural enemies on *C. medinalis* population in field is needed in order to gain a better understanding of factors that influence the population.

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