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IN-VITRO CULTURE AND DEVELOPMENTAL CHANGES OF YELLOW STEM BORER *Scirpophaga incertulas* UNDER DIFFERENT MICROCLIMATE CONDITIONS ON THE MR297 RICE VARIETY

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ABSTRACT

Scirpophaga incertulas (Walker) (Lepidoptera: Crambidae), also called the Yellow Stem Borer, is a major prominent insect pest in rice production that results in substantial crop losses. Due to climate change, the infestation of *S. incertulas* has become more serious. This study was carried out between March and April 2022 to investigate the in-vitro culture and developmental changes of *S. incertulas* under different microclimates on the MR297 rice variety. The experiment was set up in a completely randomized design, and the data on the mean developmental stages of the lengths and widths were measured. A student's t-Test was used to compare the mean differences in lengths and widths of the developmental changes in instar larvae and pupae of *S. incertulas* in the two microclimate conditions. Multi-linear regression analysis was used to correlate the relationship between the microclimate factors and the developmental changes in *S. incertulas* on the MR297 rice variety. Data were analyzed using SPSS (version 20) and R studio (version 4.2.1) software. The correlation analysis of the data showed a positive relationship between the developmental changes in lengths and widths of *S. incertulas* for the laboratory and field microclimatic conditions using the MR297 rice variety. This study successfully examined the *in-vitro* culture and developmental changes of *S. incertulas* under different microclimates, from egg hatching to the adult's emergency.

Keywords: Developmental changes, *in-vitro* culturing, microclimates *Scirpophaga incertulas*, stem borery.

ABSTRAK

Scirpophaga incertulas (Walker) (Lepidoptera: Crambidae), lebih dikenali sebagai Ulat Pengorek Batang Kuning merupakan serangga perosak utama dalam pengeluaran padi menyebabkan kehilangan tanaman yang ketara. Perubahan iklim menyebabkan serangan *S. incertulas* semakin serius. Kajian kultur in-vitro ini dijalankan antara Mac dan April 2022 untuk menyelidik perubahan perkembangan *S. Incertulas* dibawah iklim mikro berbeza pada padi MR297. Kajian menggunakan rekabentuk rawak lengkap, data min panjang dan lebar pada peringkat perkembangan diukur. Ujian T-test digunakan untuk membandingkan perbezaan min panjang dan lebar Ketika perubahan perkembangan dalam larva instar dan pupa *S. incertulas* dalam dua keadaan iklim mikro. Analisis regresi berbilang linear digunakan untuk mengaitkan hubungan antara faktor iklim mikro dan perubahan perkembangan *S. incertulas* pada varieti padi MR297. Data dianalisis menggunakan perisian SPSS (versi 20) dan R studio (versi 4.2.1). Analisis korelasi data menunjukkan hubungan positif antara perubahan perkembangan panjangan dan lebar *S. incertulas* pada iklim mikro makmal dan lapangan menggunakan varieti padi MR297. Kajian in telah berjaya mengkaji pengkulturan *S. incertulas* secara *in-vitro* dan perubahan perkembangan *S. incertulas* di bawah iklim mikro yang berbeza, dari penetasan telur kepada kemunculan serangga dewasa.

Katakunci: Perubahan perkembangan, kultur in-vitro, iklim mikro, *Scirpophaga incertulas*, pengorek batang.

INTRODUCTION

Rice is a staple food for the majority of the world's population (van Nguyen & Ferrero 2006). It is an essential and significant crop that contains gluten (8.1%), vitamins, minerals, fibre (2.2%), and many carbohydrates (77.1%), with a total of 349 calories (Alam 2013; Bisen et al. 2019). This crop is grown in at least 114 countries, the majority of which are developing countries which provide a primary source of income and employment for more than 100 million Asian households (Singh et al. 2008). Approximately 300,000 farmers are involved in rice production in Malaysia, with 150 million hectares of land set aside for rice cultivation (Hashim et al. 2017). Rice is the leading staple food for Malaysians because paddy cultivation is an economically significant agricultural activity in Malaysia regarding national food sufficiency and security (Norela et al. 2013). The Malaysian government, specifically the Ministry of Agriculture and Food Security (MAFS) is making various efforts to increase national rice production to ensure a sufficient supply for the country (Amzah et al. 2018). However, Malaysia produces 2.91 billion kg of rice annually with an average yield of 4,260 kg per hectare in the granary area which require 20% of imports to meet the demand (Dilipkumar et al. 2021). Rice production in Asia is affected by insect pest infestations such as stem borer, leafhopper, planthopper, gall midge, and grain-sucking bugs, causing an average of 20% yield loss (Kattupalli et al. 2021). The Yellow Stem Borer, *Scirpophaga incertulas* (Walker) is one of the most important rice pests, causing significant economic losses in all rice-growing regions of Asia. By inducing 'dead heart' (dead central shoot of tiller) and 'white ear' (empty ear head) symptoms, *S. incertulas* damage causes significant yield loss of up to 30% per season (Murali-Baskaran et al. 2021). Rice yield losses due to *S. incertulas* were estimated to be between 20% and 70% (Haider et al. 2021). Several studies on *S. incertulas* have been conducted, but they

lack diversity, abundance, and migration in Malaysia and other Southeast Asian countries (Yaakop et al. 2020).

The grain yield of a Malaysian rice variety (MR297) was significantly correlated with stomatal conductance, net photosynthesis rate, transpiration rate, SPAD value, leaf area index, panicles per unit area, and spikelets per panicle (Hashim et al. 2022). According to Akmal Shukri et al. (2021), Malaysian local rice varieties were solely great in combating multiple diseases, producing high yields (between 77.1% and 86.2%), and possessing good agronomic characteristics that are considered for further hybridization to achieve a broad spectrum of variation as well as the most incredible performances in agro-morphological traits. In research conducted by Ilias et al. (2020), MR 297 has the highest moisture content (3.90 0.29%), fat (22.52 0.09%), protein (12.70 0.53%), and crude fibre (3.65 0.26%), as well as the highest antioxidant activity, as evidenced by the highest total phenolic content (TPC) and ferric reducing antioxidant power (FRAP).

The microclimate is one variable that affects the insect population's dynamic (Ulfah et al. 2019). A microclimate is a localized set of atmospheric circumstances that differ from those in the surrounding areas, typically slightly but occasionally significantly, because the climate is statistical, implying geographical and temporal fluctuation in the mean values of the descriptive parameters (Chiueh et al. 2021; Hei et al. 2022). Microclimate control is critical for the optimal development of the plant, as it accounts for 90% of agricultural yields (Escamilla-garc et al. 2020). Water and fertilizer management can influence microclimate changes in temperature, relative humidity, and light intensity in rice fields by modifying the population canopy's structure, such as leaf area index, plant height, and spatial growth posture (Sun et al. 2022). According to Guotao et al. (2018), increased nitrogen fertilizer may negatively influence the microclimate health of rice cultivation by increasing the tiller number, biomass, leaf area index, and canopy. The microclimate during tillering and setting has a more immediate influence on the rice, lowering its quality (Sun et al. 2022). The microclimate can influence insect pest activity within plant ecosystems, from the canopy to the understory, and may significantly impact the distribution of pests (Grisafi et al. 2021). Pest likely uses their microclimate to find food, optimum temperature, and shelter (Rebaudo et al. 2016). Microclimatic factors such as humidity and temperature can influence the population dynamics of the invasive agricultural pest (Grisafi et al. 2021). Arthropods such as moths spend most of their cycle as larvae or pupae located in the plant or soil layers, where the temperature is in the range of their optimums (Rebaudo et al. 2016). Soundararajan (2020) studied mass culturing methods for yellow stem borer using India's susceptible TN-1 and Pusa basmati-1 rice varieties. However, no prior attempts were made to use the Malaysian MR297 rice variety to study in-vitro culture and developmental changes of the yellow stem borer under different microclimate conditions. To better understand the developmental changes of the yellow stem borer under different microclimate conditions, an *in-vitro* culture of the yellow stem borer was attempted. This study aimed to investigate the in-vitro culture and developmental changes of *S. incertulas* under different microclimates on the MR297 rice variety.

MATERIAL AND METHOD

In-vitro Culture of *S. incertulas*

The *in-vitro* culturing and developmental changes of *S. incertulas* were investigated on rice variety MR297 to assess the lengths (mm), widths (mm), and duration of the 1st instar, 2nd instar, 3rd instar, 4th instar larvae, 5th instar, and pupae under different microclimate conditions.

Sampling Techniques
Adult *S. incertulas* (male and female) were collected using a malaise trap at Tanjong Karang, Selangor, Malaysia and released into a cage measuring 18 x 30 inches (width and height) with 21-day-old paddy to allow one pair of male and female to copulate. Following copulation, the female moth lays groups of eggs on the upper surface of rice leaves.

Culturing Procedure
An experiment was conducted at the laboratory with rice leaves and a single egg; the leaves were cut to a length of about 5 cm, put in a plastic container 4 x 5 cm (width and height), and filled with prepared agar media covered with muslin cloth and a rubber band (Liao et al. 2017; Viajante & Heinrichs 1987). The eggs were exposed to embryonic development and hatching. After hatching, the first instar larvae were extracted from the egg mass using a camel hairbrush, as Manjula and Kotikal (2018) described. Newly hatched larvae were placed on healthy and fresh cuttings of rice stems measuring 6 cm in length in a plastic container 10 x 11 cm (width and height) using the same medium. These were exposed for larval, pupal, and adult development at a relative temperature of 28°C and 70% relative humidity.

A field experiment was conducted at a research farm in a cage measuring 18 x 30 inches (width and height) with ten plants per pot. The ovulated eggs of the *S. incertulas* were allowed to hatch on the plant directly, and feeding continued on freshly transplanted seedlings at 28 days old. The borer's larval stage was cultured until the adult stage, according to Mukhopadhyay et al. (2018), with a temperature of 31°C and 85% relative humidity. However, attention was given to the 1st, 2nd, 3rd, 4th, 5th, and pupae in both the study areas.

Morphometric study
Each sampling day, a random sample of 12 larvae of various sizes was taken from each research area. The morphometric larval instars, pupal lengths, and body width were analyzed using a stereo microscope and dino lite calibrated at 1.0 x magnification. *S. incertulas* larvae were fed with fresh-cut stems of rice of the MR297 variety every three (3) days during the *in-vitro* culturing under laboratory conditions until the emergency of an adult. The larvae were allowed to migrate in the shade house using the methods of Padmakumari et al. (2013) and Renuka et al. (2017). Instars were determined using the data per the Dyar (1980) rule. Briefly, the rule states that during the early stages of an arthropod's development, the size of its highly sclerotized body parts increases by a predictable and regular factor and that the length and width of a lepidopteran larva increases in a geometric progression as it grows. Daily microclimate parameters of maximum and minimum temperatures and relative humidity of morning, afternoon and evening were recorded until the pupation in both the study areas.

Statistical analysis
The experiments were conducted in a completely randomized design (CRD). The data obtained from the study were subjected to Student's t-test to compare the mean differences in Lengths and Widths of the developmental change's instars larvae and pupae in the two microclimate conditions. Multi-linear regression was used to correlate the relationship between the microclimate factors and the developmental changes in *S. incertulas*. The multi-linear regression was analyzed using R-studio (version 4.2.1) statistical computer package (Cho & Lee 2018). The software used for this analysis is R-studio.

RESULTS AND DISCUSSION

The research was conducted to study the *in-vitro* culturing, developmental changes, and time duration of *S. incertulas* and the effect of different microclimate conditions on the rice MR297 variety. The first instar larvae are yellowish-green with dark heads and exhibit solitary feeding behaviour, with mean larval lengths of 6.00 mm in the laboratory and 6.15 mm in the shade house, respectively, which is non-significant between the two microclimate conditions (Table 1) based on the t-test.

Table 1. Comparison of the mean (±S.E) developmental changes of *S. incertulas* under different microclimates

	Developmental stage	Laboratory	Shade House	*P-value*
Length (mm)	I	6.00±0.18	6.15±0.17	0.55^{ns}
	II	7.91±0.24	7.29±0.23	0.07 ^{ns}
	III	9.91±0.16	9.29±0.18	0.02*
	IV	12.96±0.22	11.54±0.44	0.01**
	V	14.75±0.27	13.85±0.30	0.04*
	Pupae	18.50±0.54	16.51±0.42	0.01**
Width (mm)	I	0.92±0.05	0.79±0.03	0.04*
	II	0.98±0.03	0.95±0.03	0.53 ^{ns}
	III	1.35±0.04	1.16±0.04	0.01**
	IV	1.83±0.02	1.69±0.06	0.05*
	V	1.89±0.02	1.88±0.02	0.81 ^{ns}
	Pupae	3.99±0.14	3.46±0.11	0.01**

Means with different p-valve across the same column differed significantly based on the T-test. * = Significant (*P<0.05*) ** = Highly significant (*P<0.01*), ns = non-significant (*P>0.0*)

The II, III, and IV instar larvae were creamy-white with blackheads and comparatively more gregarious with mean larval lengths (mm) in laboratory conditions of 7.91, 9.91, 12.96, and 7.29, 9.29, and 11.54 in the shade house (Table 1). The lengths of all developmental stages increased rapidly in the laboratory, whereas growth increased but not as dramatically in the shade house. The length at the larval stages I and II, based on the t-test, is non-significant at these stages of development. The larvae started showing significant differences from stage III, and a highly significant was recorded at larval IV instar and Pupal stages according to the t-test. The I and IV instars' mean larval width (mm) were 0.92, 0.79, 1.83, and 1.69, significantly differing as the p-value = 0.05. A highly significant score was recorded in the width of the II instars and pupae stage of the growth response of the *S. incertulus* under microclimates.

There were non-significant differences in the width of the larvae for stages II and V. The V instar larvae were dirty white, the relative mean larvae length (mm) at the laboratory was 14.75 and 13.85 at Shade house, and the mean larval width (mm) was 1.89 and 1.88, respectively. The pupae are pale to dark brown and pupate inside a silken cocoon, with a pupal mean length (mm) of 18.50 and 16.51 in the laboratory and shade house conditions,

respectively. A pupal mean width (mm) of 3.99 and 3.46 (Table 1) is highly significant (*P<0.01*).

The mean duration of the first instar larvae's development was 7.00 days, the second, third, fourth, and fifth instars were 6.17 days, and the pupal duration was 5.00 days. An increase in lengths of $r^2 = 0.98$ (Figure 1) daily under different microclimate conditions and an increase in widths of $r^2 = 0.94$ are significantly different at Shade house, whereas $r^2 = 0.90$ was observed in laboratory conditions among the various developmental stages of the larvae and pupae (Figure 2). The mean total larval period was 31 days, while the pupal period was 5.6 days in both the study areas in March and April 2022.

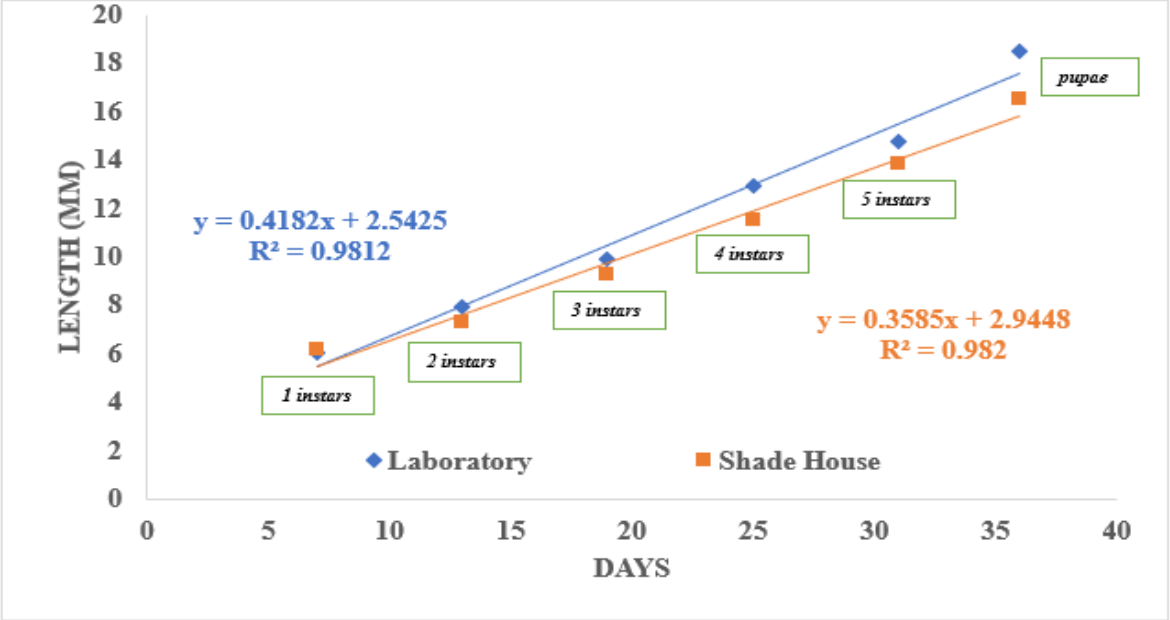

Figure 1. The *S. incertulas* growth in lengths (mm) under different development stages at different microclimates

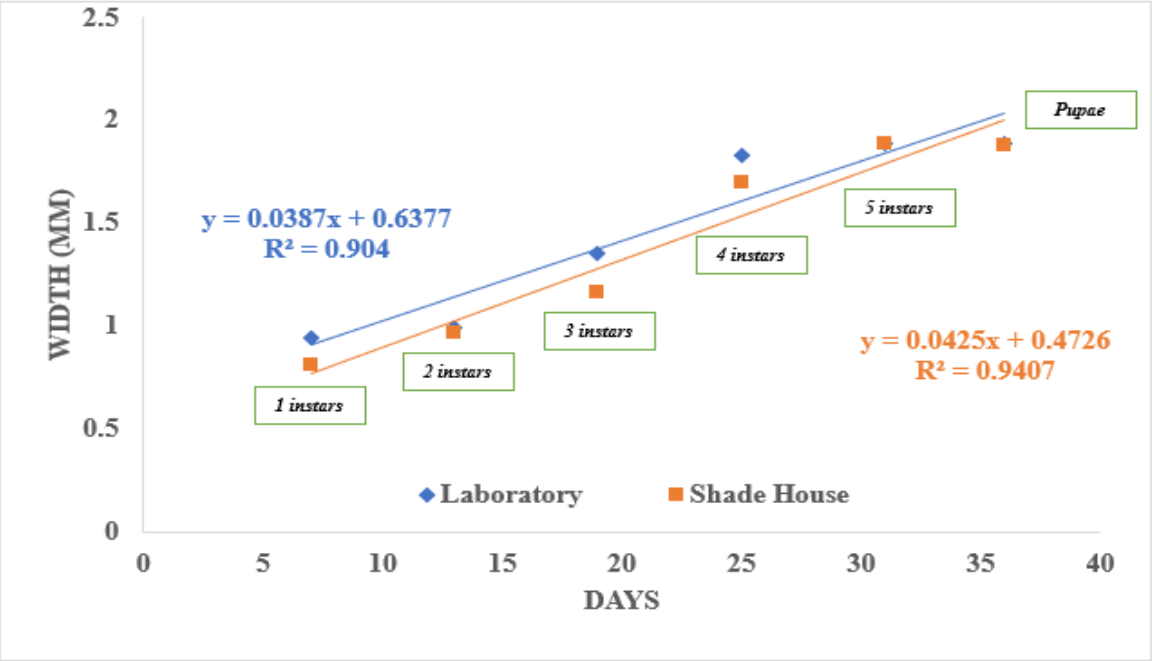

Figure 2. The *S. incertulas* growth in widths (mm) under different developmental stages at different microclimates

Multi-linear regression correlation plots of the relationship between the lengths (mm) and widths (mm) of the larval and pupae developmental stages with microclimate influence such as minimum and maximum temperatures and relative humidity. It's known that the interaction between the microclimate conditions and the host plant is very complex. The temperature and relative humidity greatly influence the development stages and number of generations of an insect within an environment. The yellow stem borer first instar larvae's lengths and widths positively correlate with the morning, afternoon, and evening minimum ºC at the shade house (Figure 3 & 4). In contrast, morning maximum ºC, RH, and afternoon minimum ºC and RH are positive in the laboratory (Figure5 & 6). At the second instar, the larval lengths and widths positively correlate with the morning minimum ºC, afternoon minimum and maximum ºC, evening minimum ºC, and maximum RH under laboratory and shade house conditions during the in vitro culturing and developmental changes of *S. incertulas.*

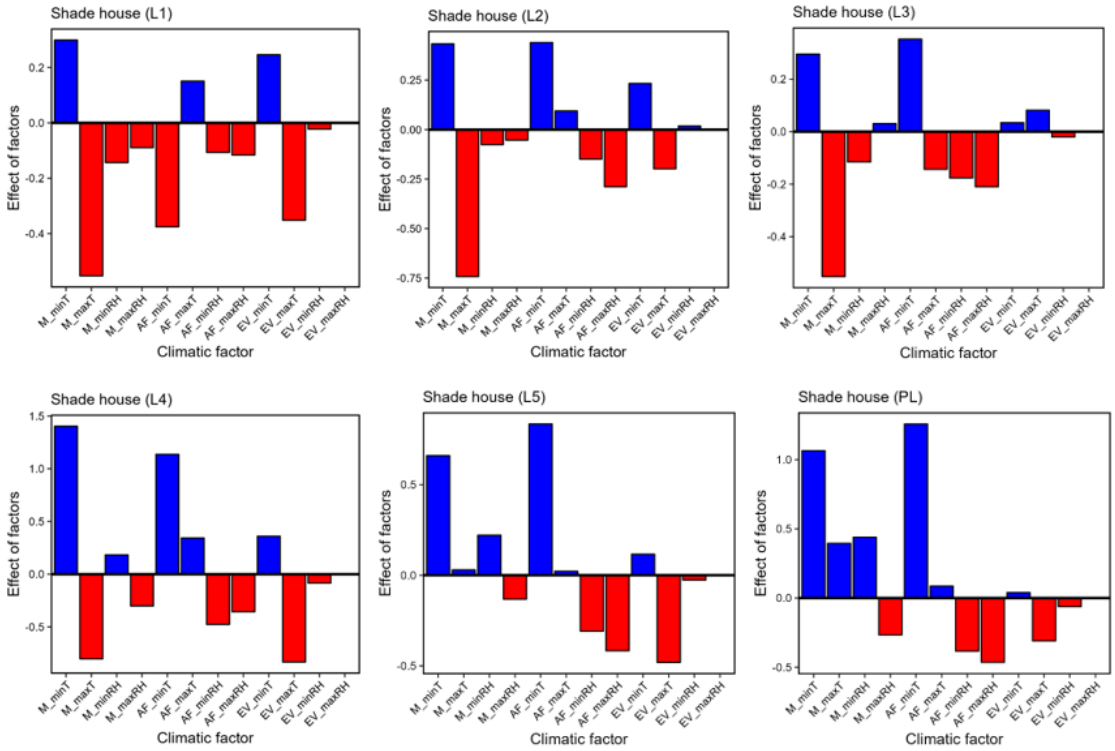

Figure 3. Multi-linear regression ggplot of the correlation of microclimate factors on the developmental changes (1st, 2nd, 3rd, 4th, and 5th instars and pupal length) of *S. incertulas* under shade house conditions

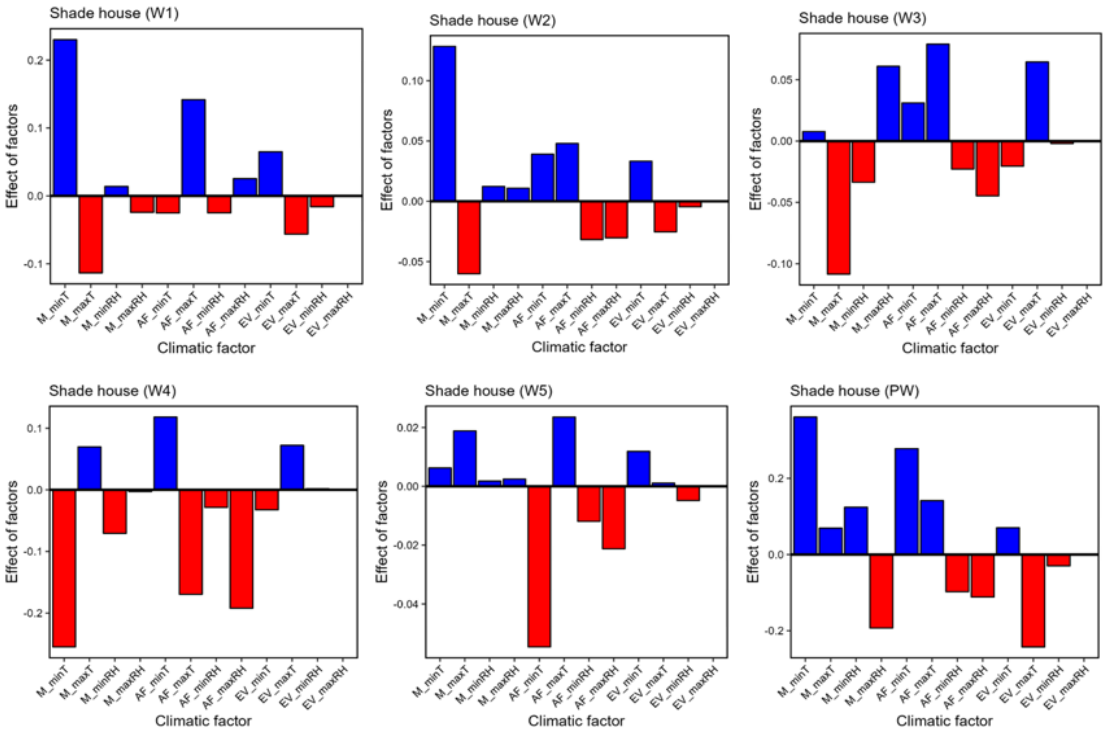

Figure 4. Multi-linear regression ggplot of the correlation of microclimate factors on the developmental changes (1st, 2nd, 3rd, 4th, and 5th instars and pupal weight) of *S. incertulas* under shade house conditions

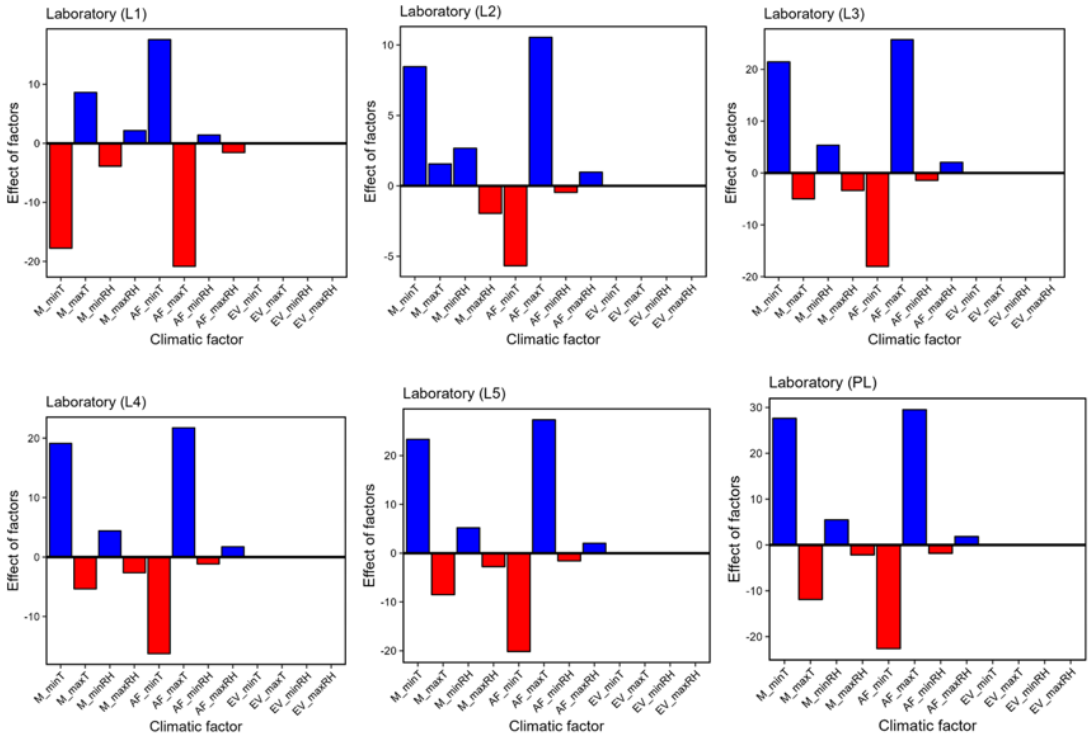

Figure 5. Multi-linear regression ggplot of the correlation of microclimate factors on the developmental changes (1st, 2nd, 3rd, 4th, and 5th instars and pupal length) of *S. incertulas* under laboratory conditions

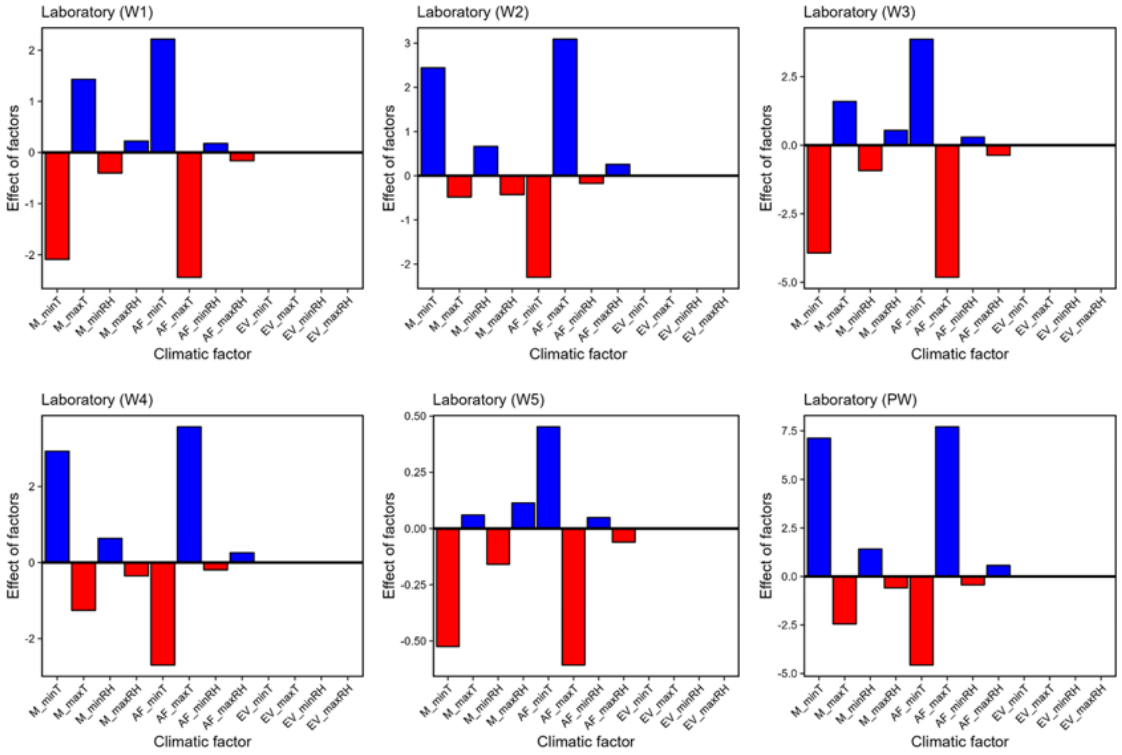

Figure 6. Multi-linear regression ggplot of the correlation of microclimate factors on the developmental changes (1st, 2nd, 3rd, 4th, and 5th instars and pupal weight) of *S. incertulas* under laboratory conditions

The developmental stages at the third instars during the larval lengths and widths measurement revealed a positive correlation with the microclimates with morning minimum C, morning maximum RH, afternoon minimum ºC, and evening minimum and maximum ºC under shade house conditions (Figure 3 & 4), contrary to the laboratory condition where morning minimum ºC and RH, maximum afternoon ºC and RH are positively correlated (Figure 5 & 6). In contrast, morning maximum ºC, RH, and afternoon minimum ºC and RH are negatively associated with the microclimate during the developmental stage in both study areas.

The lengths and widths of the fourth instar larvae were positively correlated with the morning minimum ºC, RH, afternoon minimum and maximum ºC, and evening minimum ºC at the shade house. In contrast, morning minimum ºC and RH and afternoon maximum ºC and RH are positively correlated at the laboratory condition. Morning maximum ºC, RH, afternoon minimum ºC, and maximum ºC at the shade house are negatively associated with the developmental stage, contrary to the laboratory, where only morning maximum ºC, RH, afternoon minimum ºC, and RH as recorded.

Under the shade house condition, the developmental stages fifth instar larval and pupal lengths and widths of the yellow stem borer are positively correlated with morning minimum and maximum ºC, afternoon minimum and maximum ºC, and minimum evening temperature, contrary to the laboratory condition, where morning minimum ºC and RH, maximum afternoon ºC and RH are positively correlated. Still, there is no correlation between the developmental stages with evening minimum and maximum RH in both study areas (Figure 3-6). The correlation quantifies the strength between the microclimate and the developmental changes of *S. incertulas* during the in vitro culturing process.

In-vitro Culture and Developmental Changes of *S. incertulas*

It is attainable to culture insects successfully in-vitro, and the practices involved in achieving so are helpful approaches for understanding the mechanisms involved in rearing insects (Sheeja et al. 2021). Insect culture is key to establishing, improving, and adopting a supportive approach that advances scientific investigation, insectary nutrition, and developing techniques (Huynh et al. 2021). Colony management of insects during culturing on small and large scales for the long term can impact the strain's fitness performance (Aketarawong et al. 2020). *In vitro* culturing studies, according to Parra and Coelho (2022), stated advances in nutrigenomics and multidimensional diet systems are viable for rearing *in vitro* for substantial improvement of rearing techniques for Lepidoptera. Moths can be easily reared, following standardized protocols already exist for their breeding and diet, and it has high nutritional value (Bertola and Mutinelli 2021).

In this study, the larvae and pupae of the yellow stem borer were fed with fresh rice stems of the MR297 variety, which is in line with the finding of Taylor (1996) and Bandong and Litsinger (2005), which stated that larvae feed on the parenchyma intermittent along the lumen of rice stem. Additionally, this study, for the first time, has provided a simple novel approach to rearing *S. incertulas in vitro*, as recommended by Huang et al. (2018)**,** that larvae fed on leaves of stable quality and larval development time and body size were strongly correlated, while the feeding method significantly affected larval development time and pupal weight (Holstein 2014).

The capacity to migrate and feed over vast distances while maintaining a consistent and essential steady movement trajectory is frequently found in lepidopteran insects, especially the yellow stem borer. The larva emerges from the stem whorl, encloses within the stem in a plastic

container at the laboratory, drops in itself on the field, and then bores a new hole on the fresh tender stem and rice plant. In contrast to Gao et al. (2020), migration of the *S. incertulas* is the spatial and temporal linkage of population development, as shown in the result. The first instars are small, have restricted feeding behaviour, are invariably non-motile, and do not migrate within the stem. Still, the second and fourth instars show a moderate migration with the paddy stem in laboratory and shade house conditions, while the third instar migrates actively, and the fifth instar does not. For the first time, observations on different instars of larval and pupae lengths and widths and the duration of *S. incertulus* development under various microclimate conditions are reported utilizing various study regions and the MR297 rice variety.

The larvae have taken more time to develop, which is not a favourable factor for insect developmental response, with an increase in length and width (Table 1) in line with the finding of Padmakumari et al. (2013), and the most proportional increase happened between the first and second instars. The last occurred between the penultimate and final instars. The present finding of 3rd instar larvae is significant (*P<0.05*), and the 4th instar larvae are highly significant (*P<0.01*), which is also in accordance with the findings of Gautam et al. (2020); Hugar et al. (2010); Padmakumari et al. (2013) who found that the larvae were dirty white with a length of 9.3±0.53 and 13.04±0.67 on the transplanted paddy. According to the findings of this study, there was no variation in the length and width of the fifth instar larvae, as reported by Padmakumari et al. (2013). The present findings state that the mean pupae length is significant (*P<0.05*), and the width is highly significant (*P<0.01)* compared *to* other studies. The present results revealed that there were five larvae instars and pupae observed, with each having different lengths (mm) and widths (mm), which may have been due to changes in the microclimate as several studies (Escamilla-garc et al. 2020; Ulfah et al. 2019) stated that microclimate is one of the factors influencing the dynamics of the insect's development.

Based on the above findings, the mean value of the length and width of first instar larvae is correlated with a mean duration of 7 days (Figure 1& 2), slightly different than what was reported by Satpathi et al. (2012). The present findings on the duration of the third, fourth, and fifth instars are also in the same vein as the finding of Gautam et al. (2020) 6.1 days. Figure 1 and 2 show that the mean total larval duration was 31 days, while the pupal period was five days. In line with the above, the present findings are also in agreement with the results of Hugar et al. (2010); Soundararajan (2020), who stated that the total larval duration of 32.96 days during July-September respectively, while a partial agreement with the finding of Gautam et al. (2020); Satpathi et al. (2012) as 28 days.

Influence of Microclimates on *In-vitro* Culture and Developmental Changes of *S. incertulas.*

The development, growth, and number of generations of insect species within a habitat are widely acknowledged to be highly influenced by microclimate factors, notably temperature and relative humidity (Grisafi et al. 2021; Rebaudo et al. 2016). The interaction between the many abiotic environmental factors and the host plant is intricate. In these experiments, we examine the correlations between the developmental stages and the influence of microclimate on the growth response of the *S. incertulus*, such as minimum and maximum temperatures and relative humidity, based on data collected from March to April 2022 with a digital thermohydrometer. In the field (shade house), all larval and pupal lengths and widths are positively correlated with only minimum and maximum morning temperature and relative humidity. No relationship was established between the evening microclimate factors and any of the developmental changes of *S. incertulas* (Figure 3 & 4).

The multi-linear correlation analysis of all the larval and pupal stages stated that larvae and pupal lengths and widths were positively correlated with morning and afternoon minimum and maximum temperature and relative humidity. Evening minimum and maximum relative humidity of the abiotic factors showed no relationship with respect to the length or width of the developmental changes of *S. incertulas* at laboratory conditions (Figure 5 & 6). This study is in line with the finding of Haider et al. (2021) studies, which found a substantial and positive link between *S. incertulas* population development and the minimum (27˚C) and maximum (28˚C) temperatures observed in March and April 2020. According to Ali et al. (2020), Manikandan et al. (2013), and Patel and Singh (2017), an increase in temperature will influence the physiology and development of insects and their behaviour as the number of generations changes.

As in this work, the multi-linear regression was used, which has served as a valuable tool to establish the relationship between the developmental changes of *S. incertulas* with respect to the different microclimatic environments, and to our knowledge, this is the first study to use multi-linear regression analysis ggplot to investigate the in-vitro mass rearing and developmental changes of the *S. incertulas*, specifically the first, second, third, fourth, fifth instars and pupae under different microclimate conditions.

CONCLUSION

Based on the findings of this study, in-vitro culture and developmental change of *S. incertulas* under laboratory and shade house conditions were found to be significance in addressing the developmental growth of *S. incertulas* by identifying the lengths and widths as essential parameters for appropriate identification of the larval and pupal stages. The developmental time taken by *S. incertulas* increases as insects develop faster, leading to early population growth. The correlation analysis revealed a positive relationship between the developmental changes in lengths and widths of *S. incertulas* under laboratory and field microclimatic conditions using the MR297 rice variety. This study developed an *in-vitro* culture and developmental change of *S. incertulas* under different microclimates, benefiting the agricultural industry for mass rearing, development, and integrated pest management. It recommends further research on the biology of *S. incertulas* through artificial diet feeding.

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AUTHORS DECLARATIONS

Conflict of Interest
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This manuscript has no accessible data.

Authors' Contributions
Dauda, I.: Data collection, conducting research, Statistical analysis of the data and writing the manuscript; Norida, M: Conceptualization, correspondent, data analysis, research supervision, review and editing the manuscript; Mokhtar, A.S: Reviewing and editing the manuscript, Husin, N.A: Review and editing the manuscript;

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