IDENTIFICATION AND DETERMINATION OF THE SPECTRAL REFLECTANCE PROPERTIES OF LIVE AND DEAD BAGWORMS, *Metisa plana* WALKER (Lepidoptera: Psychidae) USING VIS/NIR SPECTROSCOPY

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ABSTRACT

The bagworm is one of main and serious leaf eating insect pest threats of the oil palm plantations in Malaysia. The economic impact from a moderate bagworm attack of 10%-50% leaf damage may cause 43% yield loss. The population of bagworms without control often increases to above its threshold limits, thereby causing a serious outbreak. Monitoring and detection of the oil palm bagworm population is required to ensure proper planning of any control actions in the infested areas. Hence, a study on the determination of the spectral signature of the bagworm species of *Metisa plana* Walker was initiated by using Visible/Near Infrared (Vis/NIR) spectroscopy. Live and dead bagworm spectral properties were determined under the Vis/NIR wavelength regions, 350-1050 nm to provide specific infrared detector and band filter for development of an automated counter of the bagworms. The results showed that the live and dead bagworms had specific reflectance spectra at a specific wavelength in the NIR range spectral, ranging from 1032-1051 nm and these were statistically confirmed using the Student's t-Test with two tailed distributions. A principal component analysis (PCA) resulted that the first two principal components, F1 and F2 have eigenvalues greater than 1, at 2.11 and 1.24, respectively. Using a Boxplot Quantiles, the results showed that the lowest and highest means of the reflectance spectra observed for the live bagworms were approximately 3.43% and 26.42%, respectively. Meanwhile, for the dead bagworms, the lowest and highest reflectance spectra observed were 4.02% and 34.29%, respectively. These spectral data were important to determine the suitable infrared (IR) instrumentation for detection of every stage of the live and dead bagworms. This information will be useful, important and crucial for development of an automated detector of insect pest in the future.

Keywords: *Metisa plana*, Vis/NIR, spectral reflectance properties, spectroscopy.

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INTRODUCTION

The major insect pests of oil palm capable for causing outbreaks are bagworms. The population

of bagworms without control often increases to above its threshold limits, thereby causing serious outbreaks. There are three major species of bagworm pests of oil palm that have been reported, namely, *Metisa plana* Walker, *Pteroma pendula* Joannis and *Mahasena corbetti* Tams. In fact, the bagworms are native species that usually eat indigenous plants, nevertheless, they have changed and adapted to the hosted African oil palm, *Elaeis guineensis* Jacquin (Wood, 1976; Cheong and Tey, 2012). Norman and

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Basri (2007) reported that bagworms of the species *M. plana* and *P. pendula* are most of the cases found in Peninsular Malaysia. Meanwhile, *M. corbetti* is the most common in Eastern Sabah (Wood and Nesbit, 1969). Generally, the bagworm in the larval stage survives inside the bag or case until reaching maturity. Different species have their cases in different shapes and sizes, with variation from a narrow hose to an expansive sac (Cheong and Tey, 2012). Furthermore, *M. plana* Walker has seven larval instars or stages based on its life cycle. They create their own bags which are dissimilar in colour and size but the same in structure and shape (Basri and Kevan, 1995). Realising the importance to monitor and detect the bagworm attacks and infestation levels, the spectral properties or spectral signatures of the bagworm, *M. plana*, have been selected and found to be practical and crucial as compared to two other species of the bagworms, due to the *M. plana* species having a long life cycle and survival ability.

The selectivity and specificity of the spectroscopic approaches move towards appropriate early insect infestation detection in crops (Anielle *et al.*, 2017). Basically, the near-infrared or NIR wavelength region in the electromagnetic spectra includes the spectrum range from 780-2500 nm. Besides that, it is known that the photon energy covered under the NIR region ranges from 2.65×10^{-19} to 7.96×10^{-20} J, with wavenumbers ranging from 13 300 to 4000 cm^{-1} . Initially, the discovery of the first unseen region of light was found by German born British astronomer and music composer, Fredrick William Herschel in the 1800s. Expansion of the near infrared (NIR) spectrum application happened within three decades ago, as well as the instrumentation and spectral analysis method, by using NIR spectroscopy. This may also expand and widen the application scopes into chemical and food product analyses, chemical content determination and many others (Manickavasagan and Jayasuria, 2014). The basic fundamental of the NIR spectroscopy is based on the illumination of materials by electromagnetic emission within the NIR region. This involves the absorption of light within the NIR range by the molecules of the materials, followed by vibrations at specific frequencies relying on the chemical compound inside the materials (Murray and Williams, 1987). Based on the internal chemical interaction, an existing simple molecule grouping attached with strong interatomic bonds, such as carbon-hydrogen, oxygen-nitrogen or nitrogen-hydrogen, will generate a NIR spectrum (Manley *et al.*, 2008).

In general, the NIR spectroscopy has several advantages including fewer requirements for sample preparation and a broad scope of applications. Furthermore, the NIR spectroscopy can assist in providing important data on the chestnut

kernel or the outside part by penetrating more than 9 mm, provided there has been proper sample preparation and a suitable wavelength region (Liu *et al.*, 2011). Moreover, the NIR spectroscopy is user friendly, has a quick response, and is environmentally benign and suitable for online assessment (Pasquini, 2003; Wang *et al.*, 2010). It has been proven that this technique is effective and has met the requirements to detect insect pests or insect-damage in food merchandise and seeds. The related research works on the NIR spectroscopy are listed in Table 1 which include blueberries (Peshlov *et al.*, 2009), cherry fruit (Xing and Guyer, 2008; Xing *et al.*, 2008), fig trees (Burks *et al.*, 2000), flour products (Wilkin *et al.*, 1986), green soyabean vegetables (Sirisomboon *et al.*, 2009), jujube fruit (Wang *et al.*, 2010; Wang *et al.*, 2011) wheat products (Chambers *et al.*, 1992; Baker *et al.*, 1999), Larix species seeds (Tigabu and Oden, 2004), *Picea abies* seeds (Tigabu *et al.*, 2004), *Cordia africana* seeds (Tigabu and Oden, 2002) and other studies. In the case of insects and larvae, they are easily traced because of their internal hemolymph and/or chitin content (Rajendran, 2005) or, incidentally, due to inside damage, dryness or fungi contamination (Wang *et al.*, 2011). Besides that, the effect of different types of spectra measurements to investigate insect-damage by using Vis/NIR spectroscopy was studied by Wang *et al.* (2011) and Moscetti *et al.* (2014).

The objective of this study was to determine the spectral properties of the bagworm, *M. plana* Walker (Lepidoptera: Psychidae) by using Vis/NIR spectroscopy based on the reflectance of light at precise wavelengths in the Vis/NIR wavelength region (350-1050 nm) with the optimum intolerant capacity. The results of this study are important because they can be used to select the best IR instrumentation for bagworm detection based on their spectral reflectance properties, instead of manual observation.

MATERIALS AND METHODS

The live and dead larvae of *M. plana* Walker were collected from all the seven stages of the larval instars of *M. plana*, including the pupal stage (Figure 2). The sample size used per stage was 30 larvae/pupae, including live and dead bagworms (n=480). The larvae and pupae were collected and sampled at the smallholding areas in Mukim Chaah (2.207387, 103.038529), Johor and Tanah Mas smallholdings (4.1433155, 101.2665813) in Perak, Malaysia. The locations were varied to study the characteristic of morphology of the bagworm from different environment and insect pest management practices. Collection of the bagworm samples was specific to the *M. plana* species due to its long life cycle and survival ability. The collected bagworms were kept

TABLE 1. RELATED RESEARCH WORKS ON NEAR INFRARED (NIR) SPECTROSCOPY

Researchers	Type of insect detected	Type of food infected	Instrument used	Range of wavelength	Results
Liu <i>et al.</i> (2011)	N/A	Chestnut (<i>Castanea</i>)	Vector33 NIR spectrometer	833-2 500 nm	The assessment of chestnut quality by NIR spectroscopy. The correlation coefficients of the optimised models for sugar were 0.90 for peeled samples and 0.83 for intact samples.
Wang <i>et al.</i> (2011)	Insect, <i>Ancylis sativa</i> Liu	Jujube fruit	Handy Lambda II and Lambda NIR2.2t2	400-1 000 nm and 1 000-2 000 nm	The interactance and reflectance in the Vis/SW-NIR wavelength range exhibited higher classification accuracies in sorting severely damaged jujubes from slightly infested and intact samples.
Wang <i>et al.</i> (2010)	N/A	'Lizao' jujubes (<i>Hovenia acerba</i> Lindl.)	(Handy Lambda II and Solid Lambda NIR2.2t2	310-1 100 nm and 1 000-2 150 nm	The abilities of the interactance, reflectance, and transmission modes of visible and near-infrared (Vis/NIR) spectroscopy in detecting internal insect-infested jujubes. The highest correct classification rates obtained from the above modes were 100%, 90.0% and 97.0%, respectively.
Peshlov <i>et al.</i> (2009)	Larvae	Wild blubberies	Ocean optics SD2000	650-1 100 nm	The scanning spectrophotometers demonstrated higher signal-to-noise ratios with infestation prediction accuracies of 82% and 76.9% compared to the imaging spectrograph with 58.9% accuracy.
Sirisomboon <i>et al.</i> (2009)	Worm	Fresh green soyabean	FQA-NIR Gun	600-1 100 nm	The average raw visible and NIR reflectance spectra of good and defective pods of green soyabean showed a similar profile to absorption maximum at 650 (chlorophyll band) and 960 nm (water band).
Xing and Guyer (2008)	Larvae	Tart cherry (<i>Montmorency</i>)	FSFR FieldSpec, Analytical Spectral Devices	550-950 nm	The transmittance works slightly better than reflectance in terms of the total classification accuracy.
Tigabu and Oden (2004)	<i>Petrova perangustana</i> and <i>Dioryctria abietella</i>	Larix species seeds	1225 Infratec analyser	850-1 048 nm	A 100% recognition of infested and empty seeds in the test set by 3-factor PLS models computed based on calibration sets of each species and combined spectra of all species.
Tigabu <i>et al.</i> (2004)	<i>Plemeliella abietina</i> Seitrn (Hymenoptera, Torymidae) larvae	<i>Picea abies</i> seeds	NIR Systems Model 6500 spectrometer	1 100-2 498 nm	Discriminant analyses performed with selected NIR absorption bands also gave nearly 100% classification rate for new samples.
Tigabu and Oden (2002)	Hymenoptera, Torymidae larvae	<i>Cordia africana</i> seeds	NIR Systems Model 6500 spectrometer	1 100-2 498 nm	Possibility of using NIR spectroscopy in the seed cleaning process in the future provided that appropriate sorting instruments are developed.
Burks <i>et al.</i> (2000)	Worm and pupae	Fig trees	Diode-array NIR spectrometer	400-1 700 nm	100 passable figs and 100 figs each for the infested, rotten, sour, and dirty defect categories were examined using NIRS. Correct classifications for these varieties ranged from 83%-100%.
Baker <i>et al.</i> (1999)	<i>Anisopteromalus calandrae</i> (Howard)	Wheat products	Diode-array NIR spectrometer	400-1 700 nm	With the appropriate integration of NIR spectroscopy and seed-sorting instrumentation, kernels containing parasitoid pupae could be automatically collected from cultures of <i>A calandrae</i> during the mass rearing of this beneficial insect.
Wilkin <i>et al.</i> (1986)	Flour mite, <i>Acarus siro</i> L.	Flour products	Neotec 6350 Mk. 1 Research Composition Analyser	1 100-2 500 nm	Infested samples mite haemolymph caused the absorbance maximum for water to be shifted towards the visible end of the spectrum.

in a 500 ml cylindrical transparent plastic cage and brought back to the Entomology Laboratory at the Malaysian Palm Oil Board (MPOB) Bangi, Selangor for rearing. The live and dead bagworms were sorted out prior to being transferred into Petri dishes, each layered with a Whatman filter paper. The live and dead bagworms were sorted and determined by pinching their bags due to some bagworms were still alive but were parasitised and they were considered as the live bagworms. When pinching the bag of the bagworms, the living bagworms produced liquid at the anterior end of the bag, whilst the bag of the dead bagworms was empty and did not produce any liquid.

Identification of the spectral reflectance properties of live and dead bagworms, *M. plana*, was conducted indoors using a spectroradiometer (GER 1500, USA) (Figure 1a) based on the reflectance of light at precise wavelengths in the Vis/NIR wavelength region (350-1050 nm) with the optimum intolerant capacity. The live and dead bagworms were distinguished based on the probe signal (Figure 1b), where reflectance spectra differences were plotted for every live and dead bagworm. All sample were analysed 30 times repetition by the spectroradiometer in one round of probe detection and repeated three rounds, $n=90$. Next, the processing of the data was carried out to select the significant wavelength through a statistical analysis (Izzuddin, 2009). A Student's t-Test with a two-tailed distribution analysis was used for a visual difference interpretation and identifying those wavelengths that exhibited significant spectral separability for normally distributed datasets

between the live and dead bagworms at every stage. Data acquisition on the reflectance spectra of live and dead bagworms at every stage of their life cycle was determined. Principal component analysis (PCA), as an unsupervised method, was applied using XLStat 2017 software to quantify cluster separation at different stage of the bagworms that measured variance across all observations in the spectral data (Aaron and Michael, 2011). Boxplot Quantiles were used to compute the boxplot of five summary statistics comprised of three sample quartiles Q1, Q2 and Q3 based on the Tukey (1977) statistical analysis (Hyndman and Fan, 1996). The computation was carried out to determine whether the spectral reflectance of every bagworm stage was overlapping each other or the spectral reflectances differed significantly between all stages.

RESULTS

Different values of the reflectance spectra were observed under the Vis/NIR wavelength regions. The results of the reflectance spectra for all of the datasets are illustrated in Figures 3-10. The reflectance spectra for the live larvae of the seven instars of *M. plana* were lower as compared to the dead larvae, including the pupal stages.

From the results, PCA scores plot was used to quantify the cluster separation based on the PC1/F1 and PC2/F2 scores, which corresponded to the coordinates for the projection of each observation onto the first two, principal components. The first two principal components, F1 and F2 have eigenvalues

Figure 1. Instrument used in this study (a) a complete set of spectroradiometer, GER 1500 and (b) Spectral reflectance measurement for live and dead bagworms using calibrated probe; only one bagworm positioned on the probe during spectral measurement.

Figure 2. Image of each *M. plana* stages.

Figure 3. Spectral properties of live and dead stage 1 larval instar of *M. plana*.

Figure 4. Spectral properties of live and dead stage 2 larval instar of *M. plana*.

Figure 5. Spectral properties of live and dead stage 3 larval instar of *M. plana*.

Figure 6. Spectral properties of live and dead stage 4 larval instar of *M. plana*.

Figure 7. Spectral properties of live and dead stage 5 larval instar of *M. plana*.

Figure 8. Spectral properties of live and dead stage 6 larval instar of *M. plana*.

Figure 9. Spectral properties of live and dead stage 7 larval instar of *M. plana*.

Figure 10. Spectral properties of live and dead pupal stage of *M. plana*.

greater than 1, at 2.11 and 1.24, respectively. These two components explained 41.8% of the variation in spectral wavelength of the bagworms to separate the *M. plana* stages. Referring to Figure 10, the scores and loadings biplot showed that the larval stages 1 (0.139), 2 (0.561), 5 (0.429), 6 (0.455) and 7 (0.603) have large positive loadings on principal component, F2. Therefore, F2 focused on early and late larval stage of *M. plana*, with majority distribution of spectral wavelength from 1032, 1039, 1046, 1048 and 1051 nm. Meanwhile, the larval stages 3 (0.455), 4 (0.709) and pupal stage (0.624) had large positive loadings on principal component, F1. Subsequently, F1 focused on middle and pupal stage of *M. plana*. A statistical test, Student's t-Test, was used to evaluate the significance of the difference between spectral reflectance means and to determine statistically significant of the cluster separation in the PCA scores plot (Aaron and Michael, 2011). Besides that, the maximum difference of the reflectance between the live and dead larval and pupal stages of *M. plana* is shown in Table 2. Indeed, there was a significant difference in spectral reflectance of live ($M = 3.43$, $SD = 0.045$) and dead ($M = 4.02$, $SD = 0.043$) of first larval instars of *M. plana*, where $t(58)$ is 2.63×10^{-50} ; $p < 0.05$. The same results were observed for other bagworm stages.

The average reflectance spectra corresponding to every larval instar of the bagworm were obviously different and did not overlap with each other through the boxplot quantiles, implying that every larval instar had a specific reflectance spectra at a specific wavelength in the NIR range (Figure 11). The lowest and highest means of the reflectance spectra observed for the live bagworms were approximately 3.43% and 26.42%, respectively. Meanwhile, for the dead bagworms, the lowest and highest reflectance spectra observed were 4.02% and 34.29%, respectively (Figure 11).

DISCUSSION

Based on the reflectance spectra data, the live larvae and pupae had higher absorbance values in the NIR ranges in the reflectance measurements than the dead larvae and pupae due to the decreased reflectance of the live larval and pupal stages of *M. plana*. This contributed to more incident light to be absorbed and transmitted to the live larvae and pupae due to water content inside their bodies, which resulted in an increase in the reflectance difference between the live and dead larvae and pupae as the wavelength was augmented in the NIR range. The remitted light collected in reflectance measurements contained much more chemical and physical information about the outer surface layers of the dead larvae and pupae than about the internal tissue of the live bagworm. The same result was reported by Wang *et al.* (2010) when detecting

TABLE 2. SPECTRAL PROPERTIES OF BAGWORM, *Metisa plana* USING GER 1500, USA

Bagworm stages	Significant wavelength* (nm)	Reflectance (%)				Mean±SD		Reflectance range (%)		Sig. (2-tailed) value
		Min		Max		Live	Dead	Live	Dead	
		Live	Dead	Live	Dead					
1	1046±3	3.33	3.97	3.52	4.14	3.43±0.04	4.02±0.04	3.33–3.52	3.97–4.14	2.63×10^{-50}
2	1032±15	4.37	7.41	4.47	7.55	4.40±0.02	7.45±0.03	4.37–4.47	7.41–7.55	1.36×10^{-104}
3	1039±12	8.29	13.15	8.44	13.35	8.36±0.03	13.23±0.06	8.29–8.44	13.15–13.35	9.46×10^{-103}
4	1032±13	9.69	14.88	9.81	15.10	9.74±0.03	14.99±0.05	9.69–9.81	14.88–15.10	1.55×10^{-106}
5	1051±11	19.95	23.20	20.56	23.86	20.24±0.12	23.48±0.17	19.95–20.56	23.20–23.86	1.18×10^{-62}
6	1048±3	18.91	20.75	19.30	21.38	19.06±0.08	21.03±0.17	18.91–19.30	20.75–21.38	9.48×10^{-53}
7	1039±12	22.43	26.67	22.97	27.10	22.69±0.16	26.87±0.11	22.43–22.97	26.67–27.10	2.60×10^{-70}
pupa	1043±6	26.16	34.0	26.58	34.52	26.42±0.12	34.29±0.11	26.16–26.58	34.0–34.52	1.70×10^{-91}

Note: *The significant wavelengths are determined and validated by statistical analysis through Student's t-Test with two-tailed distributions, where $p < 0.01$. Max and min value of reflectance mode for live and dead bagworm at every stages are used to search for max reflectance difference at specific wavelength and validated using Student's t-Test. Degrees of freedom (df) for all bagworm stages is 58.

Note: Variables in red representing; 3.33 = larval stage (LS) 1, 4.43 = LS 2, 8.3 = LS 3, 9.76 = LS 4, 20.02 = LS 5, 19.03 = LS 6, 22.62 = LS 7 and 26.73 = pupal stage.

Figure 11. Scores and loadings biplot for spectral data separation; F1 vs. F2 factor scores.

Figure 12. Visualisation of different reflectance spectra for different larval instar and pupal stages of oil palm bagworms, *M. plana* by using boxplot quantiles.

infested jujubes and intact fruits in the visible/Short-wave near infrared (Vis/SW-NIR) range. Dowell *et al.* (1999) reported that wavelengths of 450-700 nm, 900-1400 nm and 1400-1700 nm were related to insect species rather than other wavelengths. Besides that, the epicuticular lipids of the insects consisted of CH_3 and CH_2 chemical components,

which are positioned at 900-1400 nm and 1400-1700 nm, respectively. Whereby, the effective wavelength for the live and dead of the larvae and pupae of *M. plana* was positioned at 1030-1050 nm. This result was aligned with Dowell *et al.* (1999) finding. Locky (1988) reported that ketones, fatty acids, aldehydes, alcohols, sterols, hydrocarbons and

glycerides were the main components of the insect cuticular lipids. Moreover, in insects, the long-chain hydrocarbons were the main composition of cuticular lipids but they could differ broadly, from 3%-95% of the overall lipid content. As an example, rice weevils contain 32% hydrocarbon of the overall lipids (Baker *et al.*, 1984). The specific wavelengths of different larval instars of *M. plana*, including the pupal stages, were obtained in the Vis/NIR ranges. This finding supported Dowell *et al.* (1999) and Lockey (1988) statements and proved that the Vis/NIR range is the correct wavelength range for all instars of *M. plana*. From Table 2, it was found that the effective wavelengths for the majority of the larvae and pupae were identified at around 1030-1050 nm and the peak around 1000 nm was generally attributed to water or OH functional groups (Wang *et al.*, 2010). According to Najib *et al.* (2018) by using the Ocean-Optics spectrometer, the maximum reflectance difference between the live and dead of the sixth larval instar of *M. plana* was detected at 715 nm. The low values in the reflectance modes for the dead sixth larval instar of *M. plana* could be ascribed to the signal-to-noise ratios that caused inefficiency to interpret the data. These two different results from the authors' current study and Najib *et al.* (2018) revealed that the bagworms could be detected by using NIR spectroscopy at definite region of wavelength under reflectance mode. The characteristic of morphology of the bagworm from different environment was not significantly affected the results because they were seem to be similar and uniform in size. This led to exhibition of same reflectance value at same region of wavelength. Furthermore, in this study, the mean of the reflectance spectra increased with the increase of the larval instar of *M. plana*, except for the sixth larval instar due to the unstable larvae positioning on the probe glass during the experiment. The surface areas of the late larval stages (fourth, fifth, sixth and seventh instar) were larger than the early larval stages (first, second and third instar), subsequently, this was attributed to the higher average reflectance spectra obtained at the effective wavelength (Table 2). According to Lockey (1988) each insect species has a cuticle with a specific and unique chemical structure, which leads to different absorbance characteristics that could be differentiated via NIR spectroscopy. Moreover, the effect of unique or specific chemical structures leads to the vibration of the molecules at specific frequencies, subsequently, resulting in the absorption of NIR energy parallel to the specific frequencies (Murray and Williams, 1987). The findings in Figure 11 generally agree with those reported by Lockey (1988) on differentiating insect species. Moreover, Siegwart *et al.* (2015) reported that the NIR spectroscopy could differentiate between *Cydia pomonella* (L.) and *Cydia molesta* (Busck) (Lepidoptera: Tortricidae)

larvae in the wavelengths between 1142 nm and 1338nm. Jia *et al.* (2014) also proved that the two species of cotton pests, *Gossypium hirsutum* L.; the tobacco budworm, *Heliothis virescens* and the corn earworm, *Helicoverpa zea* could be differentiated at early evolving stages, including egg and young larval stages, by using NIR spectroscopy, resulting in up to a 95% accuracy.

CONCLUSION

The Vis/NIR spectroscopy has been proven as a powerful instrument for quantitative and qualitative analyses which can recognise essential vibrations of the hydroxyl, amine and aldehyde functional groups within the NIR spectra. Determination of the spectral properties of oil palm bagworms is important in order to monitor and detect bagworm populations in the oil palm plantations. The reflectance spectra obtained in the Vis/NIR wavelength range using the spectroradiometer revealed that bagworms can be detected using NIR spectroscopy. Furthermore, these findings are found to be novel and provide new information in the spectroscopy technique, which can be used for inventing specific tools or devices for monitoring bagworm infestations in oil palm plantations worldwide. To meet the mission, data on spectral reflectance properties of the bagworms is used to integrate with suitable band filter specification, in terms of specific wavelength value for particular larval stages. The band filter is located or positioned on the camera lens for filtering red green blue (RGB) image and converted into infrared format, and finally searched for the targeted band of the larval instars of *M. plana* on the fronds.

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