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Measurement of damaged leaf area caused by leaffolder in rice

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Abstract
Foliage feeding insects are one of the most important guilds of pests in rice that cause significant yield losses. Rice leaffolder feeding on the foliage by scraping green mesophyll tissue leads to transparent membranous patches that are highly visible and triggers farmers to go for the insecticide application. Measurement of such type of feeding is important to know the host-pest interactions for the management of leaf feeders. In the present paper, leaf area damaged by rice leaffolder was assessed by different traditional and modern technologies like Image J program and Compu Eye, Leaf and symptom area software. The estimates had significant linear relationship with the estimates of graphical method. Of the various methods assessed for the estimation of the damaged leaf area, ImageJ software was found efficient, less time consuming and easy to operate for processing a large number of samples.

Keywords: Leaffolder, rice, damage, leaf area, estimation, method

Introduction

Rice leaffolder, *Cnaphalocrocis medinalis* Guenee is an important leaf feeding insect in many Asian Countries including China, India, Sri Lanka, Vietnam, Pakistan, Japan, Korea and Malaysia resulting in significant yield losses [1, 2]. Since 2003, occurrence of the rice leaf folder has been seriously increasing in China, resulting in infestation of more than 20 million hectare and rice yield loss of up to 760 million kg per year [3]. Similarly, in India also, increasing incidence of leaffolder was observed from different states and during 2016, severe damage (85%) was recorded in Alathur, Kollendode, Trithala, Malampuzha and Nemmara Blocks of Pattambi in Kerala and Kesamaudram Mandal of Warangal district in Telangana state [4]. Larva stitches both the edges of the leaf by silken threads and makes a fold. It feeds by scraping the green mesophyll tissue from within the fold resulting in membranous patches. These patches are usually scattered on the leaf blade due to the discontinuous type of feeding.

Measurement of such type of feeding is important to know the host-pest interactions fundamental for the management of leaf feeders. In case of rice leaffolder, damage was assessed by counting the number of damaged leaves and total number of leaves, which are then converted to per cent, damaged leaves. In some cases, the extent of damage was assessed in each leaf along with the number of damaged leaves. The extent of damage was done by visual analysis method qualitatively as area scraped of the total area [5, 6]. Though this visual scoring method is widely adopted, it has some disadvantages like subjectivity and lack of accuracy. Development of modern plant image analysis techniques [7] and Java based image processing programs [8, 9] have provided an opportunity to estimate the damaged area in a precise way. Keeping these in view, the present study was aimed to estimate and compare different traditional and modern techniques of damaged leaf area assessment methods and to identify the best method for leaffolder damage assessment so that it could be used for screening cultivars for resistance to rice leaffolder.

Materials and Methods

Twenty TN 1 leaves with different damage symptoms were used for the damaged leaf area estimation by various methods. The methods used for the estimation of the damaged leaf area include visual scoring, graphical method, leaf area meter instrument method, ImageJ software and compu Eye, leaf & symptom area software. Each damaged leaf is considered as one replication.

1. **Visual scoring method:** The damaged leaf was held in between both the fingers towards the light and based on the extent of damage and total leaf length, each leaf was evaluated and represented as per cent area damage.

2. **Graphical method:** Damaged leaf was placed on the transparent sheet and an outline of the damaged area was drawn. Area was measured by holding against a graph sheet having the smallest grid size of 1 mm. The total number of grids covered by the outline was counted. If outline occupied was more than half of the grid, it was counted as one. Based on the grid count, the leaf folder damage was calculated and represented as mm^2.

3. **Leaf area meter instrument method:** In this method, damaged leaf was placed on a white paper and outline was made and cut into leaf shape. This leaf shape along with actual damaged leaf was passed through the transparent belts of leaf area meter (Model No. LI 3100C, LI-COR, Nebraska, USA) and the LED display was noted as area in cm^2. This was converted to mm^2 for the calculation of the damaged leaf area. The difference between the cut leaf area and the actual damaged area was considered as leaf area damaged by leaf folder.

4. **Image J software:** Damaged leaves were scanned with Cannon MF 4320-4350 scanner at colour mode with 300dpi image quality. Leaf area fed was measured by using ImageJ software, an open source image processing program designed for scientific multidimensional images [8, 9]. In this software, the scanned image was opened and converted to 8-bit image. Threshold was adjusted and the damaged area was measured after setting the scale. The sliding bar of the threshold was adjusted in such a way that the whole leaf appears in red colour and the area was measured. The sliding bar was again adjusted to show the damaged area and measured after checking with actual damaged leaf. The difference between these two measurements gives the actual damaged area in mm^2.

5. **Compu Eye, Leaf & Symptom area software:** This software was developed by EM Bakr and available at http://www.ehabsoft.com/CompuEye/LeafSArea [7]. The principal idea of the software is to divide the image into small squares and assess the average colour of every square, where the area of these square units could be adjusted between 0.1 and 1.0 mm^2. The scanned leaves were opened and the colour shades of the damaged area were selected to build a detection system. In this case, we selected the transparent leaf folder damaged area many times to build "LF System". The software was calibrated using a scale before the measurement of the damaged leaf. The scanned leaf was opened in software to measure the total area and damaged area using LF detection system. The software selected the damaged area which is exhibited in red colour, and calculated as mm^2.

Results & Discussion

Estimation of the damaged leaf area by various methods revealed that the visual scoring recorded damage of 5 to 75%. Estimated damaged leaf area varied between 27 – 440 mm^2 in graphical method, 35 – 171 mm^2 in leaf area meter method, 36.6 to 447.6 mm^2 in Image J method and 34.8 to 386.5 mm^2 in compu Eye method (Fig.1). Comparison of various methods revealed that Image J method was significantly superior to visual scoring and leaf area meter estimations and was at par with graphical and compu Eye methods (Error df 76; F value 26.04 and $P \leq 0.00001$). Relationships between damaged leaf area estimated by graphical method, Image J software and compu Eye software indicated that all these methods are best suited to assess the damage. The coefficient of determination (R^2) among two sets of methods varied between 0.918 to 0.999 with highest value between Image J software and compu Eye software indicating that theses two methods can be used for assessing damaged leaf area accurately (Fig. 2).

Image J program was used to assess the damage in lettuce leaves by snails [10]. They opined that as the access to the application Image J is free, and flexible configuration allows for any setting parameters of the program, the opportunity to work in different environments and low system requirements allows making many accurate measurements in a short time without the need to purchase expensive specialized hardware. [1] A new software known as compu Eye, Leaf and symptom area was developed for measuring leaf area damaged by two spotted spider mite, *Tetranychus urticae* Koch. He observed that the software was able to assess the symptom area accurately with no significant difference compared with manual measurement.

Visual scoring method was done basically to get rough estimation of the damage. Leaf area meter estimations were very low as compared to other methods which could be mainly due to the transparent window like damage symptoms that are not clear, especially in case of feeding damage symptoms of leaffolder. Of the three methods found at par, graphical method is tedious, takes lot of time for counting and is error prone for the evaluation of large number of samples. Image J software is available in public domain, easy to operate; large samples can be estimated in less time. Compu Eye method is also available in public domain for research purpose; samples can be estimated in less time and easy to operate. This software estimates leaf damage based on the detection system which can be created by selection of colours in the damaged leaves. However, due to the variation in different leaves that are to be screened and the leaffolder damage symptoms, we could not use the same detection system for all the damaged leaves. Hence, it has taken more time for the creation of detection system for each leaf and estimation of damaged leaf area.

There are also advanced methods available that utilizes image processing techniques which helps in quick and error free measurements of pest damage symptoms (Clement *et al*, 2015; Larcher *et al*, 2015; Sehsah and Hasan, 2015) [11, 12, 13]. However, these systems need sophisticated hard ware for creating images and their analysis.

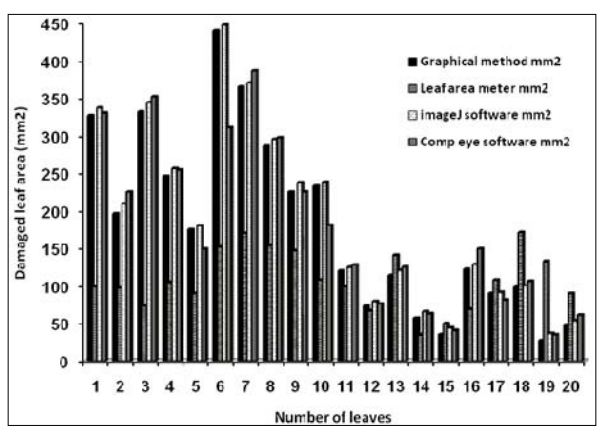

Fig 1: Estimation of damaged leaf area by various methods

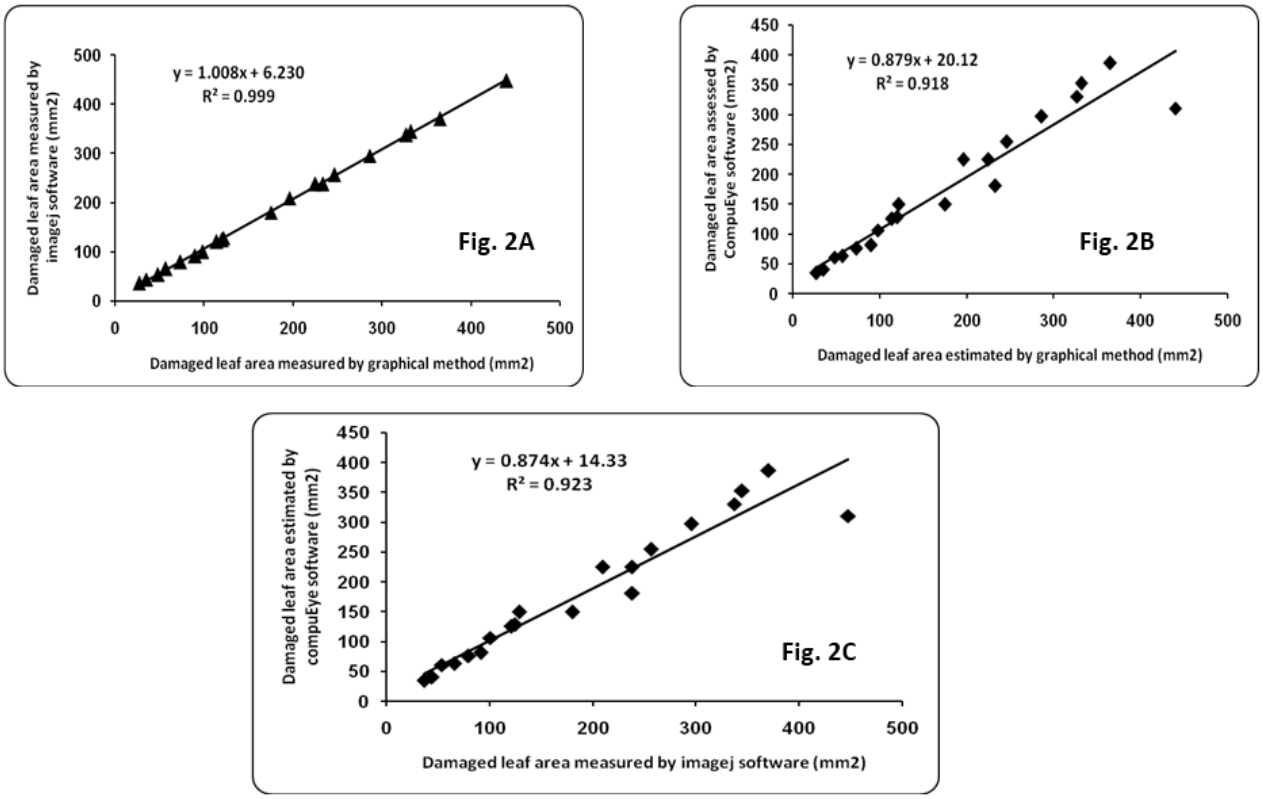

Fig 2: Relationship between damaged leaf area measured by graphical method, ImageJ program and compuEye software

Conclusions

In the present paper, leaf area damaged by rice leaffolder was assessed by different methods including traditional methods like visual scoring, graphical method, leaf area meter method and modern technologies like Image J program and Compu Eye, Leaf and symptom area software. The estimates had significant linear relationship with the estimates of graphical method. Of the various methods assessed for the estimation of the damaged leaf area, Image J software was found efficient, less time consuming and easy to operate for processing large number of samples. The quantification of leaffolder damage in the present study helps in the monitoring of populations that facilitates planning IPM activities like timing pesticide application or release of biocontrol agents.

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