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Effectiveness of different elicitors in inducing resistance in chilli (*Capsicum annuum* L.) against pathogen infection

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ABSTRACT

The excessive application of pesticides for agricultural production in Malaysia has raised quite some concern about environmental safety and sustainability. To reduce environmental impact of pesticide overuse, there is an increasing interest in using different elicitors to induce disease resistance in crop. Chilli (*Capsicum annuum* L.), which is an important vegetable cum spice crop in Malaysia, was used to compare the effectiveness of two natural elicitors (jasmonic acid and salicylic acid) with conventional pesticide application as control. The experimental results indicated that pesticide-treated plants showed rapid reduction in disease severity after application while elicitors perform slowly and its effectiveness increase gradually over time. Among the tested elicitors, jasmonic acid was found most effective regarding disease severity and yield of chilli compared with salicylic acid. Although used elicitors was not best performing treatment compared with conventional pesticide, some physiological parameters (relative chlorophyll content, chlorophyll fluorescence and photosynthesis rate) and disease severity in chilli plants treated with jasmonic acid was very close to conventional pesticide. Therefore, jasmonic acid could be a potential elicitor for inducing disease resistance in chilli and salicylic acid may not an appropriate elicitor for chilli.

1. Introduction

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is one of the most important vegetable as well as spice crops around the world including Malaysia valued for its aroma, taste, flavor and pungency. Different varieties of chilli are also being used in pharmaceuticals, cosmetics and beverages (Tiwary et al., 2005). In Malaysia, total chilli production reached about 32.8 thousand tons per year in 2010 with total cultivation areas about 2.8 thousand hectares (DOA, 2011). The total requirements of chilli in Malaysia reached up to 50 thousand tons per year and due to insufficient national production, the country need to expend a considerable amount of currency to import chilli from neighboring countries. However, the average chilli productivity in Malaysia is still very low and to achieve desirable production, it is necessary to increase both cultivation area as well as crop productivity.

Pests and disease interference is one of the major factor for lower production of chilli in Malaysia. Chilli is infested by more than 21 insects and non-insect pests. Leaf curl disease and chilli veinal mottle virus (CVMV) are most common in Malaysia (Shih et al., 2007; Arogundade et al., 2012), and some herbivores insect such as aphid, thrips and mites also acts as a vector for the transmission of plant viruses (Thaler et al., 1996).

To minimize negative consequences of pests and to maintain higher crop productivity, farmers always give priority to apply pesticides. Although pesticide application is useful to disease control, it also has some negative impacts on biodiversity and nature. Sometime farmers used huge amount of pesticides without proper diagnosis which results in resurgence of the pests, phytotoxicity on fruits, infertility and presence of high amount of pesticidal residues on harvested fruits. Extensive application of pesticides has now become another threat to sustainable agricultural production (David, 1991; Awasthi et al., 2001). Taking into consideration concerns about the sustainability of input-intensive agriculture and the economic, ecologic, and environmental effects of pesticides overuse, it is now clear that over all application of pesticide should be reduced. Agricultural scientists and planners are now giving priorities in their research for possible alternatives of the agrochemicals and pesticides.

Several studies have proved that exogenous application of different elicitors are useful to enhanced resistance to herbivore challenge and induce the expression of defense related genes

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(Lorenzo and Solano, 2005; Howe and Jander, 2008). Moreover, elicitors are also involved with different physiological activities such as seed germination, leaf senescence, stomata opening, fruit ripening and root growth (Bodenhausen and Reymond, 2007). Different elicitors are also reported to play crucial roles in plant defense responses against insect damage and microbial pathogens attack (Wasternack, 2007). Therefore, exogenous application of elicitors could be a possible alternative of pesticides application. Jasmonic acid (JA) and salicylic acid (SA) are most common plant elicitors considering disease management. Jasmonic acid is reported to increase disease resistance in plants to a variety of pests, while salicylic acid is recognized as a potential regulatory compound of different pathogens (Vigliocco et al., 2002). Although there is limited understanding of disease-resistance mechanisms of those elicitors, physiological and molecular biological studies have been documented by several authors in different plants. Using different growth parameters and disease incidence properties, we compared the effectiveness of different elicitors (jasmonic acid and salicylic acid) and conventional pesticide application in chilli plant.

2. Materials and methods

2.1. Plant material and growth condition

Mature and healthy seeds of hot chilli F1 hybrid (*Capsicum annuum* L.) were germinated in the seed tray containing peat moss and kept under protected area to enhance germination and prevent direct sunlight. Uniform seedlings were transplanted into polybag containing coconut coir dust and empty fruit bunch compost with ratio (3:1, v/v) as media substrate. Plants were transplanted individually and randomly inside a greenhouse. Plants were watered as required and fertilized with Modified Cooper Formulation two times per day during the experiment. Plants were maintained in the greenhouse under natural sunlight with 60–80% relative humidity while photosynthetically active radiation was 500–700 μmol m^{-2} s^{-1}.

2.2. Treatment applications

Jasmonic acid and salicylic acid (Sigma–Aldrich) was stored at 5 °C until needed. To prepare the spray solution, elicitors were dissolved in acetone at the rate of 1 g/ml and made up to a concentration of 1.0 mM with distilled water and 0.1% of Tween-20 was added as surfactant whereas control treatments consisted of distilled water with conventional pesticide (P) as described in Cooper and Rieske (2011). Plants were sprayed according to experimental treatments until run-off with each of the above solutions at three weeks after transplanting. Three treatments (JA, SA and P) were replicated four times and arranged into a randomized completely block design (RCBD). To avoid contamination among the treatments, each block was separated by a plastic isolator.

2.3. Plant morphology

Four randomly selected tagged plants were used to measure plant height, stem and canopy diameter. Stem and canopy diameter was determined at 100 DAT. For total leaf area determination, the area of each leaf was measured using LI-3100 Leaf Area Meter (LICOR -Inc., Lincoln, NB).

2.4. Biomass partitioning and fruit yield

After harvest (120 DAT), three randomly selected plants from each treatment were used to determine biomass partitioning in leaves, stem (including branches) and roots. The roots were gently washed under running water to remove all adhering media. Dry weight of each plant parts was measured using an electric balance (QC 35EDE-S Sartorius, Germany) and root-shoot ratio was calculated as the ratio of root dry weight and total leaf and stem dry weight. Chillies were ready to harvest while the color of fruits changed from green to intermediate reddish. Mature chillies were harvested at 5 days interval up to final harvest (120 DAT). Randomly sampled 4 chillies from each harvest were measured for fruit characteristics.

2.5. Disease incidence

The plants were monitored weekly after treatments were applied by recording the expression of disease or damage symptoms to assess disease incidence. Plant with chlorotic, necrotic, curling, mottling, stunting or bunching symptom was considered as diseases infection. The proportion of infected plants per plot was calculated and expressed as a percentage of disease incidence (Galanihe et al., 2004).

2.6. Relative chlorophyll content and chlorophyll fluorescence

Relative chlorophyll content was measured on the upper most fully expanded leaves of the four individual plants per treatment at 20, 40, 60, 80 and 100 DAT using chlorophyll meter (SPAD 502, MINOLTA™ Camera Ltd., Japan). The measurement of fast chlorophyll fluorescence was taken on the upper surface of the latest fully expanded leaves from four individual plants per treatments at 09:00–10:00 h, which was used for primary photochemistry detection. Prior to fluorescence measurements, a circular surface of the upper face was dark-adapted for 30 min using dark clips, hence the induction curve (Fv/Fm ratio) was then estimated by a Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd King's Lynn, Norfolk, England) with 600 W m^{-2} of red (630) light intensity (excitation intensity). Fv/Fm provides an estimation of the maximum photochemical efficiency or quantum yield of photosystem II (PSII) (Ouzounidou et al., 2010).

2.7. Leaf photosynthetic rate

An open-path portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, Nebraska-USA) was used to determine the net photosynthetic rate (μmol m^{-2} s^{-1}) per unit leaf area. Three uppermost fully expanded young leaves of the five individual plants per treatment were randomly chosen and measured at midday (10–11 AM). The data were taken at 20, 40, 60, 80 and 100 DAT.

2.8. Statistical analysis

An analysis of variance was performed using the SAS software (SAS, 2009). Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ was used to test differences between the treatments.

3. Result

3.1. Disease severity

Leaf curl symptoms caused by viral infection, was found as the major disease incidence in chilli plants. No significant differences were observed among the treatments at 20 and 40 DAT (Fig. 1(a)). A remarkable reduction of disease severity was observed in pesticide treated plants at 40 DAT and later growth stages. Plants treated with JA and SA showed significantly ($P \leq 0.001$) higher disease severity compared with pesticide treated plants at 40, 50 and 60 DAT. Comparing disease severity, JA was found much better than SA. Disease severity under JA and SA treated plants reduced at 80 DAT in terms