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Research Article

Analysis of Volatile Compound at Different Age of Corn Crops Used as *Bemisia tabaci* Repellent

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ABSTRACT

Bemisia tabaci is one of the red chili pests that plays a role as a Begomovirus vector. This vector can be controlled through Integrated Pest Management (IPM) practices. One of the potential practices is by utilizing corn as a barrier crop to prevent the vector from attacking the main crop. The aim of this research was to examine the repellence activity of the volatile compound obtained from various ages of corn crop against *B. tabaci.* The volatile compound was collected from the corns at 4 week after planting (WAP), 6 WAP, 8 WAP, 10 WAP, and 12 WAP. Volatile compound capture device was designed with two solvents, i.e. hexane and ethanol, then the volatile compound analyzed by Gas Chromatography Mass Spectrometry (GC-MS). *B. tabaci* repellency against volatile compound was examined using Y-tube olfactometer. Each treatment was employed with 5 replications. Data were analyzed using ANOVA with 95% and further analyzed by Least Significant Different (LSD). The parameter observed was the total of *B. tabaci* avoided the volatile compound tested until half of the control olfactometer arm. The results showed that all ages of the corn produced repellent compound and the most optimal were corn aged 12 WAP with repellency rate of 83.72%. GC-MS analysis identified the volatile compound were citronella, limonene, β-phellandrene, β-caryophyllene, 1.8 cineole, farnesol, caryophyllene, and patchouli alcohol.

Keywords: *Bemisia tabaci*, corn crop, repellent compound

INTRODUCTION

Bemisia tabaci (whitefly) is a pest that transmit a Begomovirus (yellow virus). *B. tabaci* is a very effective vector because based on greenhouse experiment within 24-hour inoculation period a single *B. tabaci* adult was able to transmit Begomovirus (the chili curly yellow disease) up to 40%, after left for 48 hours sucking the source of the inoculum. The effectiveness of transmission has doubled when 3 adults were used (Sulandari, 2004). The percentage of affected plants will increase with the increasing number of viruliferous whitefly (containing viruses) (Mehta *et al*., 1994). The virus was transmitted by *B. tabaci* persistently, which means the virus will be in the insect body as long as the insect lives. Begomovirus transmission only occurs through *B. tabaci* adult and can not be obtained through contact transmission or seeds (Aidawati *et al*., 2002; Jones, 2003; Sulandari, 2004; Hidayat & Rahmayani, 2007).

B. tabaci start indicating resistance to several types of insecticides, such as organophosphate, carbamate, and synthetic pyrethroids. For this reason, pest management based on ecological and economic approaches with Integrated Pest Management (IPM) are needed (Sugiyama, 2005; Setiawati *et al*., 2007). The development of the current and future concepts of IPM leads to bio-intensive novels, which utilize biological resources in nature, such as using natural enemies, resistant varieties, natural pesticides, repellent plants, attracting plants, or crop borders (Frisbie & Smith, 1991; Hoddle *et al*., 1998).

One of the pest control objectives in cultivation or farming is to manage the planting environment in such a way that it becomes inconvenient for pest to develop, hence reduce pest population and plant damage (Untung, 2006), including planting crop borders around cultivated crops, which are non-host plants or plants which are not preferable by the targeted pest. Non-host plants not only make the cultivated crops difficult to be found but also serve as a physical barrier for pests to find the crop. Corn plants planted around chili can reduce whitefly populations by 53% (Moreau, 2010). The use of crop borders can suppress disease incidence by viruses transmitted through insect vectors (Difonzo *et al*., 1996; Fereres, 2000). Friarini (2017) also showed that the population of *B. tabaci* in red chili plant area surrounded by corn plants was lower than that was not surrounded by corn plants.

Corn contains volatile compounds, e.g. as attractants or repellents. Attractants are chemical compounds that attract insects to come, while repellents are chemical compounds that repel insects to move away from the compound. This is confirmed by Friarini (2016), which suspected that corn has chemical compounds able to repel *B. tabaci*. Chemical compounds, including volatiles, that are terpenoids, aromatic phenols, alcohols, aldehydes, and nitrogen with molecular weights ranging from 100‒200, usually derived from secondary compounds produced by plants (Schoonhoven *et al*., 1998). Corn cob and silk has compounds such as flavonoids, carotene, quercetin, alkaloids, simple phenols (e.g. p-coumaric, saponin, tannin, anthocyanin, and protokatekin) (Guo *et al*., 2009). Therefore, this study was aimed to determine the compound content of corn at a certain age that could be used as *B. tabaci* repellents.

MATERIALS AND METHODS

The study was conducted in July 2017 ‒January 2018 at greenhouse and Entomology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. The research was carried out in two phases. The first phase was collecting volatile compounds using a volatile capture device at various ages of corn plants: 4 WAP (week after planting), 6 WAP, 8 WAP, 10 WAP, and 12 WAP. Volatile compounds were captured using two solvents, e.i. n-hexane and ethanol. The second phase was bioassay testing to *B. tabaci* using an olfactometer Y-tube. The parameter observed was the number of *B. tabaci* which avoids the tested compound. Data were analyzed using ANOVA variance and continued with LSD test with a confidence level of 95%.

Planting Corn

The corn variety used in this study was BISI-2. Fifty plants were planted in polybags (10 cm in height), and 10 plants were used for each age of corn to extract volatile compounds: 5 plants used n-hexane solvents, and 5 plants used ethanol solvents.

Mass Rearing of B. tabaci

Mass rearing of *B. tabaci* used the method by Rinaldi *et al.* (2016) with modifications: 250 *B. tabaci* adults from tomatoes planting were put inside a cage cloth (1.5 m × 1.5 m × 1.5 m) filled with tomato, eggplant, and red chili plants as host plants. Twenty host plants aged 4 WAP were placed in the cage. An infected plant was replaced with a new healthy plant. *B. tabaci* adults used in this study were from the second generation (F2) after collection from the field. Ten females of *B. tabaci* used in this study were one-day old for each treatment in each replication. The purpose of using *B. tabaci* females was referring to Hasyim *et al.* (2016), which stated that female whitefly has a higher level of virus transmission efficiency than male.

Corn Volatile Compound Collection

Corn volatile compounds were collected using a volatile compound capture device according to the method by Heath & Manukian (1994) which has been modified (Figure 1). This tool comprises of several parts: a solvent storage measuring tube, a corn plant storage tube, an air pump, and a balloon mounted on the bottom of the plant storage tube. The corn plant storage tube was designed airtight hence volatile compounds that come out from the corn plant will be captured and collected immediately. The extraction of volatile compounds was conducted by putting the same age of five corn plants into the plant storage tube. Solvents (n-hexane and ethanol) were placed alternately in the solvent storage measuring tube. The amount of solvent used was 7 ml for each collecting work. Plants that have been put into storage tube were left for one day. Thereafter, volatile compounds were captured by pumping balloon at the bottom of the tube then compounds came out, which indicated by the presence of air bubbles that infiltrated the solvent (n-hexane and ethanol). Volatile compounds collected in solvents were taken and put into small glass vials,

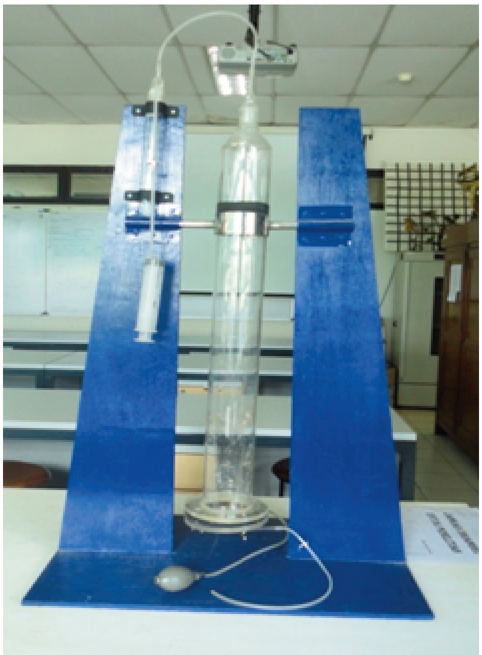

Figure 1. Device for collecting volatile compounds of the corn plant

then tightly closed and sealed using parafilm to prevent the evaporation. The caught volatile compounds were used for GC-MS analysis and *B. tabaci* repellent assay using the olfactometer Y-tube.

Corn Volatile Compound Analysis

Corn volatile compounds were analyzed using GC-MS. The experiment was conducted at the Organic Chemistry Laboratory, Department of Organic Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada. The carrier gas used was Helium with ion source temperature of 250ºC, and interface temperature of 305ºC. The obtained chromatograms were named according to the references of Wiley 229, NIST 12, and NIST 62.

Assay on the Effect of Corn Volatile Compound Repellent to **B. tabaci**

The assay on the effect of corn volatile compound repellent to *B. tabaci* was conducted according to a method by Meilin (2012). The behavior of *B. tabaci* to move away or to approach the source of the odor was performed using Y-tube (olfactometer). The olfactometer is a Y-shaped glass tube 2 cm in diameter, 10 cm in the main hand and two hands, hose, two bottles for the tested compound, an active carbon bottle, an air pump, and a flow meter. The way of the olfactometer works is: air pump runs air at a rate of 3L/minute through a bottle containing activated carbon as a filter, thus the air has passed through activated carbon does not mix with other odors. After that, the air enters the flowmeter and sets the speed of the air out to 0.3L/minute, which then passes the bottle containing the tested compound which is dropped on the filter paper. Then, air flows into the main hand of the Y tube containing the tested insects.

For each treatment, 10 drops of volatile compounds dissolved in n-hexane and ethanol as well as control (n-hexane and ethanol) or equal to 0.5 ml were dropped on the mica tube containing 1 cm × 1 cm cotton. Placing compounds with solvents was adjusted according to the combination of tested treatments. Ten *B. tabaci* adults were placed near the end of the olfactometer stalk. The number of insect entering the treatment arm or the control arm of the olfactometer was recorded (Amalia, 2015). In addition, the percentage of repellent rate was used to determine the level of repellent with the following formula (Sjam *et al*., 2010):

$$\text{Percentage of repellent} = \frac{((A\text{-}N))}{A} \times 100\%$$

A: number of adults in control

N: number of adults in the solution of the compound of corn plants

Level 1 = repellent value between 0.1–20%

Level 2 = repellent value between 20.1–40%

Level 3 = repellent value between 40.1–60%

Level 4 = repellent value between 60.1–80%

Level 5 = repellent value between 80.1–100%

B. tabaci chose stimulating compounds in one of the Y tube hands (Amalia, 2015). *B. tabaci* was allowed to choose and waited for 10 minutes to walked to the 7 cm Y tube hand from the base and stayed for one minute at that location. If *B. tabaci* did not elect any of which within 10 minutes, it was deemed to have no response (Meilin, 2012). The parameters observed in this experiment were the number of *B. tabaci* which avoids volatile compounds of corn plants at various ages.

Data Analysis

In this study, if *B. tabaci* did not choose the arm containing corn compound it means that the insect avoided stimulating from corn compound. Data were analyzed with ANOVA and continued with the LSD test at 95% confidence level.

RESULTS AND DISCUSSION

The olfactometer assay showed that the number of *B. tabaci* moving towards the control arm (n-hexane) was significantly different from the treatment on the arm containing extract of corn plants. In the control, *B. tabaci* was less repelled compared to the treatment of plant age. The corn plant that most repelling *B. tabaci* was age 12 WAP (8.6) and age 8 WAP (8), where insects preferred to moved toward control (n-hexane). As for treatments using ethanol, the result showed that the control was significantly different from other treatments of corn plants from various ages. In control, *B. tabaci* adult was only slightly repelled compared to the treatments. It was considered that in the treatments with volatile compounds from various ages found compounds that were able to repel *B. tabaci*. The highest number of *B. tabaci* repelled by volatile compounds with ethanol was in aged 12 WAP (8.6), which were not significantly different from volatile compounds aged 10 WAP (7.8). Whereas at age 10 WAP (7.8) there was no significant difference with volatile compounds from age 8 WAP (7.2). From treatments above, the most repelled number of *B. tabaci* was at the age of 12 WAP. The use of n-hexane and ethanol solvents showed that both treatments have the same plant age that produced the highest repellent to *B. tabaci* (12 WAP) (Table 1).

The GC-MS analysis of volatile compounds of several ages of corn plants, which dissolved in n-hexane and ethanol solvents, detected several compounds which in some previous studies were mentioned as insect repellents (Table 1). Monoterpene compounds have a rejection effect on mosquitoes including α-pinene, cineol, eugenol, limonene, terpinolene, citronellol, citronellal, camphor, and thymol. Likewise with sesquiterpenes, such as β-caryophyllene (Nerio *et al*., 2010). Citronellol and geraniol are resistant to *B. tabaci* adults (Delletre *et al*., 2015).

In this analysis, patchouli alcohol was also detected, which in cymbopogon and eucalyptus extract acts

Table 1. The GC-MS analysis of volatile compounds of corn plants at various ages with n-hexane and ethanol solvents and repellent assay to *Bemisia tabaci*

Plant Age (WAP)	Detected Repellent Compound		Repellence of *B. tabaci* (% ± SE)*	
	n-hexane solvent	ethanol solvent	n-hexane solvent	ethanol solvent
4	Geranyl acetate	Bornyl acetate	55.95 ± 6.63a	46.43 ± 8.50a
6	-	Citronellyl acetate	36.19 ± 10.50a	47.62 ± 5.83a
8	Limonene Citronella Citronellol acetate Eugenol Geranyl acetate β-Caryophyllene Farnesol	Patchouli alcohol	74.21 ± 5.04a	59.52 ± 7.67a
10	1,8-Cineole Farnesol	Champene Myrcene β-Phellandrene Citronella Caryophyllene β- Farnesene α-Patchoulene	64.29 ± 4.37a	70.63 ± 6.06a
12	1,8-Cineole Farnesol	β-Patchoulene Caryophyllene β- Caryophyllene α- Patchoulene Patchouli alcohol	83.33 ± 3.40a	83.33 ± 3.40a

Remarks: *Values ± SE followed by the same letter were not significantly different according to LSD (α = 0.05) as insect repellents (Mardiningsih *et al*., 1995). Likewise, citronella, cyclohexene, β-citronella, β-phellandrene, and citronellyl acetate which in orange peel served as repellent compounds and could bring mortality to termites (Noverita *et al*., 2014). In addition, citral compounds (geranial and neral), geraniol, citronellol, citronellal, piperitone, linalool, elemol, 1,8-cineole, limonene, geraniol, β-caryophyllene, methyl heptenone, geranyl acetate, and geranyl contained in cymbopogon essential oils have been known to serve as antibacterial, antifungal, anti-yeast, insecticide and insect repellent for a long time (Ganjewala, 2009). *Tithonia diversifolia* is a plant can be used as a vegetable insecticide. *T. diversifolia* acts as antifeedant and anti-oviposition for whitefly (Susanti *et al*., 2015) which contains essential oils in the form of α-pinene, σ-pinene, and limonene compounds (Oladipupo *et al*., 2012).

From the assay on the effect of corn volatile compound repellent to *B. tabaci*, there was no significant difference between volatile compounds which dissolved in n-hexane and ethanol. This showed that volatile compounds were able to dissolve in n-hexane and ethanol. There were similarities in volatile compounds found in some corn ages which were soluble in n-hexane and ethanol, including citronella and β-caryophyllene. In addition, there were 1.8 cineole, farnesol, β-patchoulene, caryophyllene, α-patchoulene, and patchouli alcohol. These compounds have a high level of repellency to *B. tabaci*, hence these compounds considered be used as a repellent compound for *B. tabaci*.

CONCLUSION

Corn plants produce volatile compounds that are repellent to *B. tabaci*. Volatile compounds of corn found has a role as a repellent to *B. tabaci* were citronella, limonene, β-phellandrene, β-caryophyllene, 1.8 cineole, farnesol, caryophyllene, and patchouli alcohol. Corn with the highest repellency level using n-hexane and ethanol solvents were at the age of 12 WAP (8.6). The suggestion from this study is to protect the main crop, especially chili from *B. tabaci* as a yellow virus vector. Therefore, planting corn as a barrier should be preceded before the main crop. It is recommended to plant the main crop when the corn plant has reached 4 WAP.

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