

The Efficacy of Companion Crops Planting for Controlling Virus Transmission in Sweet Potato Cultivation

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Abstract

Background: Sweet potato (*Ipomoea batatas* L.) is well known nutritious and healthy source of food that reported vulnerable to viral diseases worldwide including Malaysia. Management of pest vector that responsible for the transfer of virus disease is really important in sweet potato planting. Aphid transmits Sweet potato feathery mottle virus (SPFMV) meanwhile whitefly transmits Sweet potato chlorotic stunt virus (SPCSV). SPFMV when singly or mix with other viruses had caused reduction in the quantity and quality of the sweet potato tubers.

Objectives: Aims of this study is to control the pest vector using plants compounds with pesticidal effect to repel pests.

Materials: Chives (*Allium tuberosum*) were planted in companion to sweet potato as a prophylactic measure to manage the pest vectors responsible for the transfer of virus disease at farmer plot in Semenyih, Selangor. A total number of 192 bags of chives were planted companion to sweet potato in a ratio 1:2 as repellent crop. Partial budgeting on the effect of sweet potato planting with chives was evaluated.

Results: Sweet potato planted with chives showed lower mean number of aphid and whitefly when compared to mean number for aphid and whitefly in the control plants (sweet potato without chives). A lower virus incidence percentage was also significantly observed in sweet potato planted with chives (27.5%) compared to the control plants (41.2%). Moreover, sweet potato grown with chives attracted more beneficial arthropod/ insects.

Conclusion: In conclusion, planting sweet potato with chives has a positive impact on the production with 13% increment of sweet potato yield compared to planting sweet potato alone. Farmer earned extra income from the sale of chives in addition to the existing sweet potato produce. Furthermore, results of the analysis showed that more positive benefits were obtained with the value of net income RM 344.57 when sweet potato was planted companion with chives.

Keywords: Aphid; Chives; Sweet potato chlorotic stunt virus (SPCSV); Sweet potato feathery mottle virus (SPFMV); Whitefly

Introduction

Sweet potato is a superb source of vitamins, minerals, anti-oxidants, dietary fiber, protein and calcium. All parts of sweet potato plants are edible and it is easy to grow. In Malaysia, sweet

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potato is well known in food industry as a source of nutritious and healthy diet. According to the Malaysia crop statistic data in 2019, sweet potato was cultivated highest in the state of Perak (1,846 ha) followed by Kelantan (596 ha) and Selangor (267 ha) [1]. Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV) is the most important virus reported affecting sweet potato crop worldwide. The combination of SPFMV and SPCSV is known as sweet potato virus disease (SPVD) where in this case, the viruses may help each other to multiply resulting more severe symptoms of infection in sweet potato. In Malaysia, the virus attack was first discovered in 2009 [2]. By 2016, an area of 63 hectares of Vitato cultivation in Tembila and Dendong Kelantan were attacked by virus of which caused reduction to the farmer's yield and income. An average income of farmers decreased from RM30,000 / season to RM10,000 / season [2]. A wide range of aphid species mainly by winged adults transmits SPFMV in non-persistent manner. By other means, SPFMV could also be transmitted through the use of infected cuttings materials [3]. SPCSV is transmitted by the whitefly especially *Bemisia tabaci*, as they fly from plant to plant by feeding up the plant sap to cause many plants become infected. Whitefly usually is the driving force behind the spread of SPVD. Once a virus enters a plant cells, it will take over part of the management of the cell's processes. These new virus particles will then spread to adjacent cells, increases the permeability of plasmodesmata to virus particles resulting accumulation of carbohydrates and decreasing of photosynthesis rate in the leaves [4]. The common virus symptom in sweet potato is chlorosis of the leaf tissue either between the leaf veins or along the veins. The leaves could be misshapen with an uneven or curled appearance. Pigmented leaves, often purple or yellow spots or rings could also be observed. Moreover, virus infection will affect the yield quality of sweet potato and reduce its storage roots production and volume of harvest. It is inevitable that infected sweet potato multiplied on grower's farm over several seasons will lead to the reduction of sweet potato grower's income. It is really important to control the pest vector of sweet potato viruses. To mitigate the effect of virus, farmers normally practice a scheduled spraying of insecticides to control the vector population. However, the use of agricultural chemicals (insecticide) to the target plants would eventually affect the whole site including crop plants, soil organisms, humans and wildlife in that area. As an alternative, much attention has been focused on eco-friendly methods to control the virus and its vector. Generally, virus disease could be managed via immunization and prophylactic measure. Application of repellent companion crop for the management of pest in prophylactic measure is well documented. Plants with pesticidal properties possess compounds that have effects to repel pests. The botanical compounds with pesticidal activity that successfully have been isolated and commercialized including azadirachtin from neem (*Azadirachta*

indica), pyrethrin from pyrethrum (*Tanacetum cinerariifolium*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis*), ginger (*Zingiber officinale*) and thyme (*Thymus vulgaris*) [5]. *Allium spp.* volatiles was found able to inhibit attraction of aphid to a host plant [6]. In this study, the important compounds of chives were determined through chemical analysis. The efficacy of applying chives as pest repellent crop with sweet potato was evaluated with the target to reduce the number of pest population that vectored virus disease in sweet potato cultivation. Moreover, the economic impact using partial budgeting was calculated for the expected change in profit of having chives planted companion to sweet potato.

Materials and Methods

Plot and planting materials

This study was carried out at farmer's farm in Semenyih Selangor in July to November 2020. Virus-free sweet potato (variety Lembayung) cuttings that collected from MARDI Bachok Kelantan were planted in polybags contained of coco peat media at hot spot area of farmer's farm in Semenyih Selangor. Chives that propagated in a glasshouse at MARDI Serdang were used as a companion crop in this study. Chives that planted at ratio 1:2 (chives: sweet potato) two weeks prior to sweet potato planting were used as a treatment in this study meanwhile, sweet potato planted without chives were used as a control. This experiment was arranged in completely randomized design.

Leaf gas exchange parameters

Leaf gas exchange parameters of sweet potato were measured using a Portable Photosynthesis System (LI-COR 6400, LICOR Inc., U.S.A.) at 70 days after planting. Photosynthetically active radiation (PAR) of the leaf chamber was set at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The chamber temperature was maintained at 30°C and the reference CO₂ concentration was 400 ppm ($\mu\text{mol mol}^{-1}$). Relative humidity was controlled between 50-70% with airflow rate at 500 $\mu\text{mol s}^{-1}$. The gas exchange measurements were done on the mature, unshaded and completely expanded leaves; between 0800 to 1100 h, which photosynthetic rates would be maximal [7]. The leaf gas exchange for net photosynthesis rates (A) ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of virus infected and healthy plants were recorded and statistically analysed.

Monitoring of pest vector and beneficial insect in sweet potato cultivation

Monitoring of pest vectors (aphid and whitefly) and beneficial insect were conducted using scouting, yellow sticky trap (YST) and sweeping methods for three times throughout the planting season. In each monitoring; pest vector was monitored using ten random scouting/ treatment, five YST/treatment and five sweeps /

treatment. Scouting was done by direct counting of the presence of aphid and whitefly on ventral side of five apical leaves in ten random plants. YST was placed at appropriate boundaries in the plot and collected after 24h. The arthropod caught on each YST and sweeping were identified and classified by their Order and Family. Mean number of aphid and whitefly population in sweet potato planted with chives and control were evaluated and statistically analysed.

Monitoring of incidence and identification of virus in sweet potato cultivation

Virus incidence was scored for three times throughout the planting season. The presence of virus symptoms on sweet potato leaves grown with chives and without chives were recorded quantitatively. Disease incidence percentage is calculated following the equation:

Disease incidence percentage = (Symptomatic plant/ whole plant population) X 100

Virus identification process that involve of RNA extraction and cDNA synthesis was carried out using HiScript II One step RT-PCR kit (Vazyme) following the manufacturer's manual. Polymerase chain reaction (PCR) was performed using specific primers for different viruses. The PCR products were then cloned and sent for sequencing analysis. A derived DNA sequence of the main virus was registered in National Center for Biotechnology Information (NCBI).

Statistical analysis

The data generated from monitoring of pest vector, beneficial insect and virus incidence were subjected to statistical analysis using one-way ANOVA without interaction (SAS 9.4). The means significance differences for pest vector and disease incidence of sweet potato planted with chives and control were then performed using Duncan's multiple range tests (the minimum significance was set at $P < 0.05$).

Chemical compound analysis of chives

Chemical compound analysis of chives was carried out using five grams of powdered sample of chives that mixed with 45 mL methanol at a ratio of (1:9). The samples were continuously shaken for 7 hours at 250 rpm and subsequently filtered with a filter paper no 1 (Whatman) into a conical flask. Two mL methanol extract of chives was then transferred into a GC vial and injected into a GC-MS. The GC-MS instrumentations consisted of a gas chromatograph mass spectrometer (GC/MS) analysis performed on an Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector. The column used was DB-5MS UI (Agilent Technologies) with 30m length, 0.25 mm diameter with a

stationary phase of 5% phenyl methylpolysiloxane, 0.25 μm film thickness. An identification of peaks were done by a MSD Chemstation to find all of the peaks in a raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0. Selective compounds for sulphide components as active compounds were determined.

Partial budget analysis

This study had employed partial budget analysis towards chives planting for controlling virus transmission in sweet potato cultivation. Partial budgeting is a consistent method for calculating the expected change in profit from a proposed change in the farm business without determining the absolute profit. The essentials parameter that taken into account includes additional cost incurred, lost or reduction of current income, additional income received and reduction or elimination to the current cost. The analysis done using this partial budgeting was adapted from [8].

Results and Discussions

Identification of virus

SPFMV was identified as the major virus infecting sweet potato plants in farmer's field in Semenyih Selangor. Other than that, SPCSV also was also observed in the farmer's field with virus incidence less than 5%. Leaves infected by SPFMV showed of purple ringspot (Figure 1-i) and yellow chlorotic (Figure 1-ii). Meanwhile the symptom of SPCSV symptom is observed having purplish stippling between the veins (Figure 1-iii). The major virus presence was confirmed through PCR analysis of which produced a band size approximately 400bp. Analysis of the DNA sequencing product showed a 98.8% similarity to Sweet potato feathery mottle virus in the tested samples.



Figure 1: Symptoms of SPFMV in sweet potato: purple ring spot symptom (i) and yellow chlorotic symptom (ii). Symptom of SPCSV: purplish stippling between the veins (iii).

The DNA sequence of major infecting virus (SPFMV) was registered in NCBI under accession MW255529. Virus infection reduced the number of roots formed as well as the diameter of the roots resulting in a greater length to diameter ratio compared to the non-infected plants. This could be contributed by low photosynthesis rate in the symptomatic leaves of the infected

plants. Based on the evaluation in this study, photosynthesis rate was found to be significantly lower in symptomatic leaves of virus infected plants when compared to the healthy (virus free) plants. This finding is supported by the result of who explained cassava root yield losses was determined from viral infection that caused photosynthetic alterations associated with changes in chloroplast ultra-structure and carbohydrate metabolism of cassava plants [9] (Figures 1 and 2).

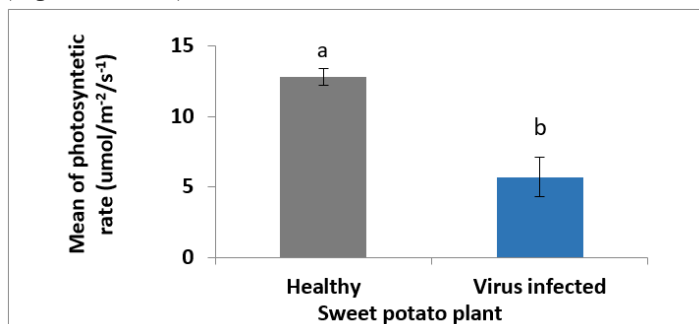


Figure 2: Mean of photosynthetic rate in healthy sweet potato (virus free) compared to infected sweet potato plant. Means covered by the same letter are not significantly different.

Pest vector and beneficial insect population in sweet potato cultivation

Aphid and whitefly were observed at all interval of monitoring. Analysis revealed no significant difference ($P > 0.05$) of mean number for aphid and whitefly population between sweet potato planted with chives or control plants. Nevertheless, a lower mean number of aphid (3.67 ± 1.9) and whitefly (74.1 ± 28) in sweet potato planted with chives were observed when compared to mean number of aphid (7.56 ± 3.1) and whitefly (102.0 ± 34) in the control plants. It is important to control virus incidence by lowering the pest vector at low population as aphid could transmit SPFMV in a non-persistent manner of which means aphid uptake the virus (acquisition) within just a few seconds on the infected plant and directly transmits the virus to a healthy plant. On the other hand, whitefly normally has short flight between neighbouring plants and rarely flying more than 0.5m above the canopy to spread the virus [10]. Selection of intercropping plant with potential effects on the

target pest is important to ensure the success of eliminating virus transmission by pest vector. Volatile organic compound from *Allium tuberosum* was studied as an alternative to chemicals for pest control [11]. A study found a lower densities of *B. tabaci* and/or incidence of associated viruses when tomato were intercropped with *Allium spp* [12]. Preliminary screening of a hexane-chives extract using GC-MS, identified three sulfide tentative compounds as dimethyl trisulfide (2.974%), S-methyl methanethiosulfinate (2.204%) and S-methyl methanethiosulphonate (1.744%) in chives.

Similarly, sulfide compound was detected in *Allium spp* [13] by as an important pest repellent. Sulfide in chives would make insects to make oriented movements away from its source. Sulfide in chives may react chemically and physiologically with the host plant making it unsuitable host for aphids [14]. Moreover, chives that remain green throughout the year in tropical conditions would continuously releasing volatile organic compound to provide persistent protection [15]. In addition, sweet potato grown with chives also attracted significant number of predators in Order Diptera (Family: Dolichopodidae). Other than that, predator in Order Araneae (Family: Oxyopidae and Lycosidae) and parasitoid in Order Hymenoptera (Family: Platygasteridae, Eulophidae, Chalcididae, Braconidae) were also observed higher in sweet potato grown with chives compared to the control.

Virus incidence in sweet potato cultivation

The virus incidence percentage reported in this study was based on SPFMV and SPCSV symptoms such as mottle, mosaic, stunted, ring spot and purplish stippling between the veins of sweet potato leaves. Mean of the virus incidence shown in Figure is significantly ($P < 0.05$) lower in sweet potato planted with chives (27.5%) compared to the control plants (41.2%). The lower incidence of virus disease in sweet potato planted with chives is hypothesized correlated with the lower number of aphid and whitefly population observed in this study. Most importantly, sweet potato planted with chives produced 13% more yield than the harvest of sweet potato without chives. Similarly, [12] found a higher yield of tomato when it was intercropped with *Allium spp*. compared to plots grown with tomato alone (Figures 3 and 4) (Table 1).

Table 1: Sulphide compounds identified in a crude extract of *Allium tuberosum*.

Sulphide Compounds	GC-MS Library Matching (%)	Relative GC-MS Area (%)
Trisulfide compound		
Dimethyl trisulfide	95	2.974
Thiosulfinate compounds		
S-Methyl methanethiosulfinate	70	2.204
S-Methyl methanethiosulphonate	94	1.744

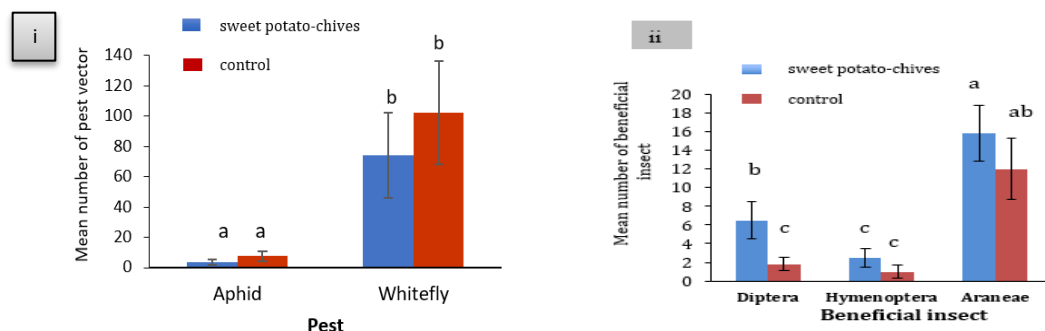


Figure 3: Mean number of pest vector in sweet potato cultivation (i). Mean number of beneficial insect in sweet potato cultivation (ii). Means covered by the same letter are not significantly different.

Table 2: Partial budget result for companion crops planting in sweet potato cultivation.

Proposed change: Companion crops planting for controlling virus transmission in sweet potato cultivation			
Positive effects	Value	Negative effects	Value
Additional income:		Reduced income	
Purple sweet potato (13%)	447.00	-	-
Companion crop	377.57		
Total additional income	824.57	Total reduced income	-
Reduced costs:		Additional costs:	
-	-	Companions crop seed (192*RM 2.50/polybag)	480.00
Total reduced costs	-	Total additional costs	480.00
Total additional income and reduced costs (TAIRC)	RM 824.57	Total reduced income and additional costs (TRIAC)	RM 480.00
Change in net income (TAIRC-TRIAC)			RM 344.57

Partial budgeting on the effect of sweet potato planting with chives

Partial budget is suitable approach in assessing changes in the business with associating with the specific change in the farm [16-18]. According to partial budget comprises of four categorical parts of which additional income, reduced costs, reduced income and additional costs. Farmers normally dealt with changes and adjustment in farm management due to maximizing profit, enhancing size or improvement in some technical aspect. The farm price for sweet potato was RM 12 per kilogram when data of the yield was collected in November 2020. Results of the partial budget analysis in Table 2 evaluated that the net impact of planting chives as companion crops for controlling virus transmission in sweet potato cultivation was having positive effect. The total of positive effects was RM 344.57/acres/season. Meanwhile, the negative effects only involved the additional costs of chives at RM

2.50 per polybags for 192 seedlings. Furthermore, findings of this study revealed a positive effect on change in net income resulted from small changes in the farm management.

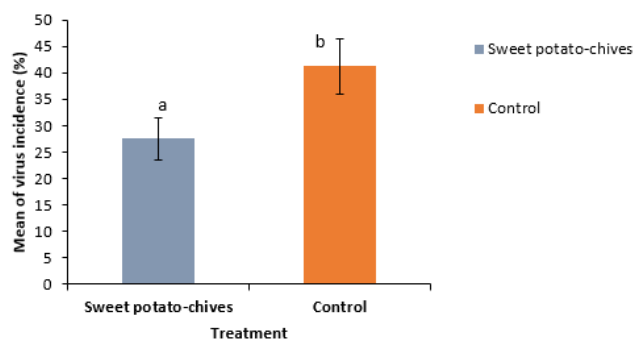


Figure 4: Mean of incidence percentage of virus disease in sweet potato cultivation. Means covered by the same letter are not significantly different.

An additional income was contributed by production of sweet potato at RM 447.00 and revenue for chives as companion crops at RM 377.57. The extra earn by farmer in this study is supported by who mentioned that profit maximization are drivers for farmers motivation for the expected profit or expected utility maximization [19] (Table 2).

Conclusion

Planting chives as a companion crop in sweet potato cultivation showed an effective method to control the virus pest vector. A reduction of pest vector population in sweet potato planted with chives would make a significant different reduction of virus incidence. Furthermore, chives that known to have sulphide active compounds could be planted throughout entire period for years to benefits farmers generating an additional income. The findings however would be repeated for more seasons to verify the results in the near future.

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