

Effects of Using *Ocimum* spp. as pest Repellent Plants on Chinese kale (*Brassica Oleracea* L.cv. *albolabra*) in Dry Season Condition

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ABSTRACT

This study was carried out to assess the effect of repellent plants on insect population and damage on Chinese kale. The experiment was designed in randomized complete blocks design (RCBD) with four treatments and 4 replications, 16 plots. The treatments using various types of basil, sweet basil (*ocimumbasilicum*), lemon basil (*ocimumbasilicumcitriodorum*), holy basil (*ocimumtenuiflorum*) while plots without barrier were used as a control treatment. Spring onion was intercropped in all treatments. Insect sampling was done on 10 plants/plot selected by W-line to observe weekly intervals of pest occurrences. Average numbers of each species were recorded. The two main pests infested Chinese kale were aphid (*Brevicoryne brassicae*) and Diamondback moth (*Plutellaxystella*). Moreover, the accumulative number of pests over time was significantly different among treatments and the mean of the total population was therefore statically different. The lowest insect number was found in sweet basil and holy basil treatments while serious damaged leaf area resulted in the control treatment. The findings revealed that the most effective basil in reducing infestation levels of aphid was sweet basil while holy basil shown promising results in suppressing DBM. The evaluation could help keep up a sustainable Chinese kale production by beneficial insect enhancement and free chemical application. Despite basil could have been integrated in managing Chinese kale, crop's yield was not significantly different.

Keywords: Accumulative number; Infestation; IPM; Intercropping; Repellent plants; Rates

INTRODUCTION

Chinese kale (*Brassica oleracea* L.cv. *albolabra*) is a leafy vegetable crop which is originated in China. The Chinese kale is classified into the same species as common kale, common broccoli, cauliflower and head cabbage, brassica oleracea, but it is in the cultivar group *albolabra* [1]. It is therefore called Chinese broccoli, Kailan, or Gai-lan [2]. The production is generally decreased drastically due to pest devastation and diseases. Jamornarn reported that applying pesticide to control pests becomes a key method for farmers and however it is a consequence when synthesis pesticides resulted in unintended and unforeseen problems, not only creating insect resistant to insecticides, but also bring up the secondary pest outbreaks and polluting the overall environment [3]. Aside from polluting and

developing resistant generations, pesticide is possible to eliminate beneficial insects and other non-target organisms [4]. More importantly, the increasing application of insecticide will cause a health hazard to consumers due to long persistent of residues, innate toxicity, and bio magnification of the pesticide residues in the human body over a period of time [5]. Cambodian farmers have applied many pesticides and mixed around 4-6 kinds of pesticide in a tank to protect their crops. Consequently, the chemical pesticide cocktail applied is a cause of polluting the environment and leading agriculture to unsustainability. Seriously, some farmers have given up their cultivation due to the fact that they have no choice besides using chemical substances to control the outbreak of pests [6]. Around 16 insect species have been reported on cruciferous vegetables in Cambodia [7,8]. At least 22 insect pests have been identified on

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this crop in Cambodia and only five are the major pests [9]. Among these, the main insect pests are: *Plutellaxylostella*, *Brevicorynebrassicae*, *Hellulaundalis*, *Phyllotretastriolata* and *Spodopteraexigua*. *Plutellaxylostella* is the most serious pests on cruciferous around the world [10]. Planting pest repellent plants (PRP) is a useful method which can decrease or suppress the abundance of insect pests [2]. Sweet basil (*ocimumbasilicum var. pilosum*), lemon basil (*ocimumbasilicumcitriodorum*) and holy basil (*ocimumtenuiflorum*) contain Linalool oil which is recognized as insect repellent oil. It could repel diamondback moth, flea beetle, armyworm, cabbage webworm in Battambang province [11] and Royal University of Agriculture [9]. There were some specific insects had identified to be repelled by these basil such as aphid, Japanese beetles, vegetable weevils and spider mites [12]. Growing repellent plants is an alternative method preventing insect in organic practice, in terms of avoiding synthesis pesticides application, growth regulators, livestock addictive, etc. Palaniappa and Annadurai (1999) and Farlex (2004). Supawankianmateeand s.l.ranamukhaarachchi (2007) indicated that Chinese kale is sensitive to insect pest required a large amount of pesticide to control, hence pest repellent plant might be a good use [13-15]. One more thing intercropped brassica crops with spring onion (*Allium fistulosum*) could repel flea beetle, armyworm, diamondback moth and cabbage webworm in Battambang province [9,11] flea beetle, armyworm, diamondback moth in Kandal province [16]. More importantly, intercropped with spring onion can maximize the yield of brassica crop [17]. Midmore illustrated that intercropping is an efficient use of water, nutrients and solar energy which surely can increase crop productivity compared with growing a single crop type [18]. In term of soil conservation, growing intercropping is an efficient way to improve soil quality by wasting residue of course, ground cover and different rooting systems of two varieties of the crop can reach to various depths [3].

METHODS AND MATERIALS

The Randomized Complete Block Design (RCBD) was arranged for the replication and randomization of treatments. The plots were designed by four treatments and four replicates giving a total of four experimental rows. The experimental treatments were: control treatment, Sweet Basil, Lemon Basil and Holy Basil, and those treatments were intercropped with spring onion.

Cow manure was applied as based fertilizer which spread over on the plots about 20days before transplanting seedlings. This experiment was applied urea (46-0-0) for additional grown promotion. The recommendation rate was 112.5g mixed with 60 liters of water on 30 m². All treatments executed the fertilizer rate equally for 2 times of crop life cycle [19,20]. A week after transplanting the first data sampling took place and the following collections were made on 7 Days after transplanting (DAT). A manual collection was in W shape [21,22]. 10 plants/block were randomly selected in W shape 7 days interval; hence 40 samples were recorded in each treatment and 160 plants were sampled per time. The population of insect was assumed to be stable. The numbers of insect per 10 plants were accumulated through weeks to figure out the daily variables. Each replication

was inserted in to scatter in order to settle linear formulation $y = ax + b$ then found out "a" value in the four replications of each treatment (a: the slope of line, b: is the interception). The correlation of insects with infested leaf area was calculated by paring the data of each insect and dependent variables (infested leaf area and unmarketable yield). R square (R²) was figured out by entering the dependent and independent variables and shown R-squared value [23]. The 10 samples collected from a block used in order to figure out the average weight per plant; "W" shape sampling was randomized selected 3 rows in the middle of plots and 10 plants were selected. The parameters included: Leaf area was accessed by selecting 3 leaves (third to the fifth leaf) per plant. 5 plants per plot were collected, 15 leaves per plot which accounted to be photographed. Huawei Nova 2i's camera which attached 16-megapixel (4608×3456 pixels) was installed by keeping 50 cm space from the camera to the background of leaves. The background of leaves put a white color paper so that Photoshop could distinguish leaf area and its background. A centimeter ruler grid was laid down extensively on the paper nearby the leaf then exported to the Photoshop CC. Firstly, leaf must be imported to Photoshop CC then identified the length and pixels by Image>Analysis>Set Measurement scale in other to transform pixels to centimeter. Then Leaf was imported in the Photoshop clipboard, click on Magic wand tool then click on Add to selection mode in order to identify leaf area until the selection surrounded the area. After the bordering line surrounded leaf fully, go to Image>Analysis>Record measurement to figure out area shown in Measurement log dialog box. Infested leaf area analyzed by magic tool by clicking on selection mode in order to spot on holes and go to Record Measurements to save damage leaf area in Photoshop CC [2]. Marketable yield and unmarketable yield and total yield were also collected. Data collected were analyzed by using the STATISTIX 8 to test the differences in means (ANNOVA). Mean separation was done using Least Significant Differences (LSD) at $P < 0.05$ [24].

RESULTS AND DISCUSSION

The mean of total pests collected from 7 Days After Transplanting to 49 Days After Transplanting (DAT) was 7times, a weekly interval to observe the population changes on each treatment of 40 samples. There were several types of pest which pressured on Chinese kale throughout the growing season such as flea beetle (*Phyllotretastriolat*), aphid (*Brevicoryne brassica*), diamondback moth (DBM-*Plutellaxylostella*), Beet armyworm (*Spodopteraexigua*) and Large Cabbage-heart Caterpillar (*LCHC-Crocidolomiabinotalis*). However, repellent effects of various basil with spring onion intercropped were observed to be reduced the number of Aphid and DBM respectively (Table 1). There were statistically significant differences among the treatment. Using sweet basil as a pest repellent plant greatly decreased the number of aphid presented in 44.11 of 10 plants while growing holy basil as a protective barrier performed lower in the mean total number of 159.11. However, without the barrier and cropping lemon basil found the highest presence of aphid. Meanwhile, DBM recorded the lowest number in holy basil 24.25 along with lemon basil barrier 30.75 of total pest

which was similar to sweet basil 38.25, but the control treatment observed tremendously different from the other basil.

Jeffery 2002 mentioned that the changes of climatic condition expand the population of pests, particularly temperature. Despite Jeffery, Capinera (2012) and Soth (2017) reported that the number of aphids affected by climatic changes and seasons. The aphid is commonly active in dry season and most of adult are females that could be generated new generations of offspring very quick. According to the Department of Water Resource and Meteorology 2018, reported the temperature was increased highly from January to March (33.7-35.5°C) that could possibly provide a perfect condition for aphid to produce more generations. Comparably, the accumulative of aphid shown the drastically moved up from 7 DAT to 49 DAT, it indicated that during the experimental period (January to February) mean of

temperature was 34.63°C which stimulated the reproduction chances and developed significantly up to 4.01 rate accumulated overtimes in control treatment while lemon basil treatment reached to 7.79 of rate (per day).

Table 1: Mean of total pest of 10samples/plot in each treatment collected from the 7 weeks (7DAT-49DAT)

High value of SE in each treatment in this study might because of the pattern of insect distribution naturally are not homogeneous enough, ie replicates on the edge were likely to be attached by insects, while plots in the middle were less. Evaluation of different types of repellent plants, using a greater number of replication, perhaps bigger plots could facilitate more robust.

Treatments	Insets population, mean/10 plants				
	Flea beetle (<i>P. striolata</i>)	Aphid (<i>B. brassica</i>)	DBM (<i>P. xylostella</i>)	Beet armyworm (<i>S. exigua</i>)	LCHC (<i>C.binotalis</i>)
Control	24.5 ± 6.4	258.24 ± 13.16 a	45.25 ± 2.3 a	24.38 ± 7.2	12.7 ± 21.54
Sweet basil	4 ± 6.4	44.11 ± 13.16 c	38.25 ± 2.3 ab	22.37 ± 7.2	30.7 ± 15.23
Lemon basil	70.5 ± 6.4	303.33 ± 10.74 a	30.75 ± 1.89 b	5.88 ± 7.2	28.1 ± 21.54
Holy basil	14.00 ± 6.4	159.11 ± 13.16 b	24.25 ± 2.3 b	18.38 ± 7.2	15.5 ± 21.54
F %	11.9	71.76	12.75	0.93	0.16
LSD(p=0.05)	ns	**	*	ns	ns

7 DAT: 7 Days after Transplanting, 49 DAT: 49 Days after Transplanting, SE: Standard error of mean, Ns: non-significantly difference, *significantly different at 95% confidence level, ** significantly different at 99% confidence level, F: F test value, LSD: Fisher's Least Significant Different Test, CV: Coefficient of Variation, Different letters in A B C D are the significances in treatments

Rates of insects accumulated over time: the effects of repellent barriers resulted differently on rates of each insect overtimes collected from 10 plants in each block. There was numerous insect's rate presented throughout the days on Chinese kale like Flea beetle (*Phyllotreta striolata*), Aphid (*Brevicoryne brassica*), Diamondback moth (DBM-*plutellaxylostella*), Beet armyworm (*Spodoptera exigua*) and Large Cabbage-heart Caterpillar (LCHC-*crocidolomiabinotalis*)(Table 2). Regarding the table, there were statically significances among the treatments. Aphid was different as statistical analysis shown the highest rate per day of 7.79% (10 plants) in lemon basil treatment followed by 3.59% of holy basil and similar to control treatment had 4.01%of rate. However, the lowest accumulation rates of aphid found in sweet basil as a rate of 2.05%. Moreover, DBM was also significantly different presented largely in no barrier treatment (control) carried 0.88 of rate per day while DBM existed less number on sweet basil treatment with 0.75. Anyway, in the lemon basil treatment, only 0.54 rates occurred while the lowest number instant in holy basil block. Contractedly, flea beetle, beet

armyworm and LCHC were not disparate even though planting the barrier.

From December to February the DBM population was hatched out greatly compared to duration from March to May [16]. This harsh condition happened during the experimental process that caused large presence of DBM due to favorable climatic condition. Moreover, the relative humidity dropped rapidly during the experiment providing a good environment for duplicating population during the study. General climatic condition like temperature, high humidity and rainfall are proper for aphid to be conducive for the population growth [24]. Sweet basil (*Ocimum basilicum*) was reported its effectiveness in reducing aphid infestation levels by its aromatic smell [25]. Sweet basil has a strong scent that deters pests by forcing them off and the odor of this herb. Furthermore, the research conducted by Seagraves declared that sweet basil has essential oil accommodated biologically-active constituents that are insecticide, nematocidal and fungistatic or which have antimicrobial properties. Those properties might have imputed to excessive essential oil elements such as methyl chavicol and methyl cinnamate which can dismiss insect pests, therefore, reducing their populations in fields [26]. Holy basil also has its biological properties similar to sweet basil that could have been effective in repelling aphid. Lavacidle activity is strong compared to other species. This activity reported the effectiveness by disturbing and killing lava of pest, particularly DBM. DMB has

been widely known as the main pest devastates crucifer crops [10]. Intercropping by only spring onion in the field may not be an effective method to control pests. As a result of the experiment, control treatment used spring onion without barriers had found profusion mean population of DBM while using basils reduced the number of DBM respectively (Table 1). Sweet basil contains oil elements such as linalool, eugenol, geraniol and methyl eugenol that are reproductive agents in repelling pests [27]. On the other hand, cropping using sweet basil as a repellent plant with strong aromatic smell could depress insects such as cabbage webworm and DBM etc. [28]. Holy basil therefore classified in the same family to sweet basil. Main constituents of holy basil are methyl chavicol, eugenol, and eucalyptol with other constituents being α -humulene,

humulene-epoxide II, (-)-trans-caryophyllene, α -trans-bergamotene, and γ -cadinene [29], those compositions could have been released strongly with various functions that would be much more better than using sweet basil. Khun, Hok, Dara revealed that sweet basil could diminish DMB 45%, flea beetle 23%, beet armyworm 82% and cabbage webworm 75%. According to the total population of beet army worm, it had no differences between barriers and without barriers. It was opposite to Khun's finding, sweet basil was the most effective to reduce beet armyworm population up to 82%. This compatible result may have been caused by the number of pests occurred in largely different numbers in each replication of the treatment [30].

SE: Standard errors of mean, Ns: non-significantly different, ** significantly different at 99% confidence level, F: F test value, LSD: Fisher's Least Significant Different Test, CV: Coefficient of Variation and Different letters in A B C D means significances among treatments.

Treatments	Accumulated number of insects, rates/10plants				
	Flea beetle (<i>P. striolata</i>)	Aphid (<i>B. brassica</i>)	DBM (<i>P. xylostella</i>)	Beet armyworm (<i>S.exigua</i>)	LCHC (<i>C. binotalis</i>)
	Rate(per day)	Rate(per day)	Rate(per day)	Rate(per day)	Rate(per day)
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Control	9.25 \pm 2.01	4.01 \pm 0.19 b	0.88 \pm 0.03 a	1.96 \pm 0.81	0.43 \pm 0.32
Sweet basil	6.13 \pm 2.02	2.05 \pm 0.15 c	0.75 \pm 0.02 b	1.60 \pm 0.70	0.67 \pm 0.32
Lemon basil	8.05 \pm 1.56	7.79 \pm 0.19 a	0.54 \pm 0.02 c	1.11 \pm 0.70	0.46 \pm 0.31
Holy basil	0.87 \pm 1.53	3.59 \pm 0.15 b	0.46 \pm 0.02 d	1.3 \pm 0.70	0.08 \pm 0.24
F%	4.5	130.67	67.06	0.23	0.68
LSD(p=0.05)	ns	**	**	ns	ns

Crop data/parameters: Insect pests infested on Chinese kale fed on different parts of their favorite flavors and places. Regarding the mouth part of those insect pests like flea beet, chewing mouth part feeding on young leaf while diamondback moth consumed green tender leaf in the downside. Likely to beet armyworm and Cabbage-heart caterpillar use its mouth part to chew young leave as well; however, aphid which was found in the experiment was outstanding on sucking flavor from leaf tissue causing serious damage. Hence, the pests mostly provoked badly on leaves that could not be sold in the market, therefore it reduced total yield. According to the analysis of applying diverse plant barriers and free barrier planting could have been different in mean of damaging (Figure 1). Graph indicated total leaf area and damaged leaf were measured in centimeter square (horizontal axis) tested in the four treatments (vertical axis). Total leaf area was not significant different even though repellent barriers was growing, yet the damage leaf area shown significantly differences. Without planting barrier was the highest damage in Least Significant Differences test (LSD) followed by holly basil and lemon basil barriers. However, sweet basil plots performed greatly to reduce damage compared to

other treatments. Marketable yield and unmarketable yield were calculated by Kg per m² depending on the size of plots of each treatment. There were no significant differences between marketable yield, unmarketable yield and total yield of Chinese kale.

Damaged leaf area represented in no barriers treatment was extremely infested. It could have been resulted by population outbreak due to the fact that aphid had the highest population while Diamondback moth presented minor at the same treatment. The development of pests had the correlation with the quality of its host leaf. Pest prefers feeding on young, tender or soft leaves yet reduces consuming ability on old leave. Chinese kale production has a strong relation between population and foods, and actually aphid was overpopulated

due to food quality [28].

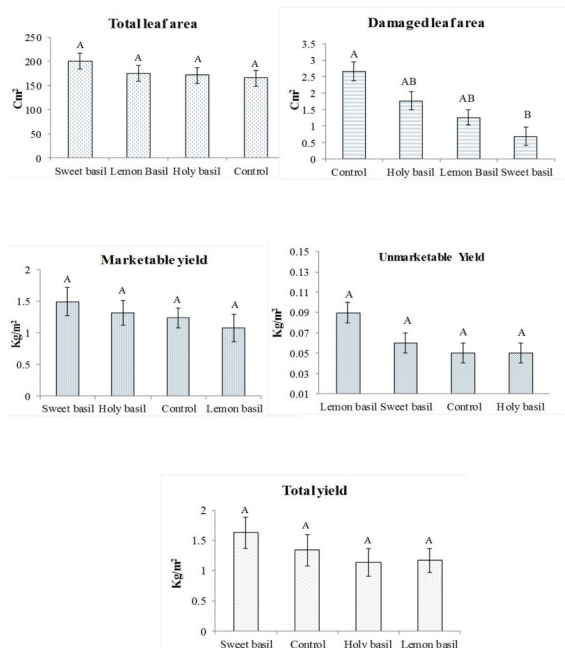


Figure 1: Graphs and statistics of the mean of crop parameters which y: total leaf area (cm²), infested leaf area (Cm²), marketable and unmarketable yield (Kg/m²) and total yield (Kg/m²) and x: treatments. SE was assessed by analyzing the variance of linear models and different in letters was figured out by LSD All-Pair wise Comparisons Test.

CONCLUSIONS

Aphid and diamondback moth were considered as the major pest due to its overpopulated host vegetable and beet armyworm, large cabbage-heart caterpillar, and beet armyworm were the secondary pests. Climatic changes were exactly the factors of drastically increasing the pest populations and the accumulative number of pests (rates per day). Sweet basil was productively in repelling aphid while holy basil had strong potential in controlling diamondback moth. Without IPM barriers cropping, Chinese kale leaf was seriously infested by pests while the population of those insects was not effective on yield component. Hence IPM barriers could only repel the main pests however; it could not a key factor in maximizing Chinese kale yield. In term of environmental conservation and human hazardous harms, this experiment was successfully completed as there was not even a small amount of pesticide applied to control pests.

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