The Journal of Agricultural Sciences - Sri Lanka
Vol. 17, No 3, September 2022. Pp 360-369
http://doi.org/10.4038/jas.v17i3.9917

Potential of Kaffir Lime (*Citrus hystrix*) Leaf Extract as a Biopesticide in Improving pre and post-harvest Quality of Chili (*Capsicum annuum*)

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Received:27th December 2021 / Accepted: 06th July 2022

ABSTRACT

Purpose: *Chilli production in Malaysia is declining due to production problems such as pest and diseases which cause plants to become stunted, unhealthy, and damaged. To overcome this problem, most farmers prefer using chemical fertilizers and pesticides due to their fast reaction and effectiveness. In long term, application of these chemicals input may lead to an environmental damage and human health problems. To conserve our nature, the use of natural input in agriculture is highly recommended.*

Research Method: *This study was conducted to identify the potential of plant extract, namely kaffir lime in increasing the plant productivity and reducing the severity of pests for chilli trees. The study was performed by spraying the chilli trees with 25, 50, and 75% (v/v) kaffir lime extracts. Controls consisted of plants without any treatment as negative control and plants applied with a commercial chemical pesticide as positive control.*

Findings: *Results indicated that there were significant differences ($p<0.05$) between treatments with respect to all parameters including pre-harvest (plant height and pest severity) and postharvest (fresh weight and number of chilli pods) parameters except for the stem and crown diameter, soluble solid concentration, fruit firmness and colour measurement of chilli. The results found that chilli plants sprayed with 25% kaffir lime extract had the lowest pest infestation. Moreover, application of the 25% kaffir lime extract had produced the heaviest weight of chilli fruits and the highest number of chilli pods compared to other treatments including the positive control (chemical pesticide). Therefore, it was concluded that 25% kaffir lime extract produced healthier chilli plants, higher yield and better quality of chilli.*

Originality/ Value: *Limited number of studies are available on effectiveness of kaffir lime-based insecticides. Therefore, this study was conducted as an effort towards understanding the versatility of kaffir lime leaves extraction as an alternative pesticide on chilli plants' pre- and postharvest performance.*

Keywords: *Biopesticides, Chilli, Kaffir Lime, Pre-harvest, Post-harvest, Quality*

INTRODUCTION

In Malaysia, chilli is one of the major crops with a year-round high demand. Chilli is also a cash crop and can be planted throughout the year. It is intensively cultivated in three states of Malaysia that include Kelantan, Johor and Pahang with cultivation area of 536, 469 and 399 ha, respectively (DOA, 2019). Chilli is one of the important ingredients used in many dishes of Malaysians. It can be used either raw (green or red), cooked or processed for the preparation of other foodstuffs. Total chilli production in Malaysia was 47, 015mt in 2015 but decreased sharply to 43,738mt in 2016 (DOA, 2019). The production was slightly increased to 26,354mt in 2019. However, this amount is still insufficient to meet the local demand mainly during festive

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seasons. Malaysia has to import 39% of chillies from Thailand and Vietnam to fulfil this demand.

Pest and diseases are among the main causes in the declining of chilli production. It is worsening as the chilli plant itself is very susceptible to pests and diseases (Touhidur *et al.,* 2006). The chilli plant can be infested by more than 21 insects and microbes (Dey *et al.,* 2001). As a result, the tree becomes stunted, damaged, and finally dies. Currently, most farmers go for chemical pesticide as a solution to pest and disease problems. A large number of chemical pesticides, insecticides, and fertilizers were used to boost crop production. It resulted in successful harvest and production during the early stages of application, but during the last four decades, the soil's productivity has declined (Vanita *et al.,* 2014). Despite the growing popularity of organic farming, conventional pesticides are still being used in the agriculture practice and for sanitation purposes; thus, pesticide residual occurrence in environmental matrices is still a concern (Srimurali *et al.,* 2015). Although chemical pesticides have a reputation as an effective and rapid effect, regular and continuous use will have a negative impact on human health and the environment. The use of Excessive pesticide resulted in destroying habitats. The health of many birds, marine plants, and livestock is threatened by toxic pesticides (Mahmood *et al.,* 2016). Overuse of toxins in marine environments has been noted, posing a potential danger to fish populations such as salmon. In addition, the farmers and macro-invertebrates are also affected by pesticides (Macneale *et al.,* 2010).

One of the solutions to reduce the usage of chemical pesticides is Integrated Pest Management (IPM) principles, which prioritize physical and biological regulation techniques (Munier and Dongmo, 2010). It is a cross between traditional farming, which uses a lot of inputs, and organic farming, which does not use any industrial pesticides or fertilizers. Another alternative is sustainable agriculture where the cultivation of fruit, fibre, and other plants or animal products cultivated in an environmentally sustainable manner using various farming techniques. In this concept, conservation of biodiversity in the soil is important for agriculture's long-term viability.

Recently, many studies related to plant extract as biopesticides have been conducted. Plant extracts are well known to contain a high toxicity level towards a large array of insect pest (Saleem *et al.,* 2019). Plant extracts can inhibit insect growth and development, result in significant weight loss in larvae, pupae, and adults. They also prolonged the larval and pupal stages, as well as the time it took for pupae to heal and adults to emerge (Khanam *et al.,* 1990). They might be used as repellents, feeding deterrents, poisons, or hormone mimics to prevent enemy attraction (Odeyemi *et al.,* 2008), oviposition, and growth regulation (Rozman *et al.,* 2007). Hence, it can be concluded that botanical products are helpful and desired tools in most pest control programmes because they may be effective and frequently complement the actions of natural enemies, degrade quickly in the environment, have low human toxicity, and reduce the possibility of pest biotype selection (Greenberg *et al.,* 2005).

Based on the above facts, the use of bio-pesticides is very appropriate. However, there are some challenges in applying this method in the crop production process; i.e. lack of confidence among farmers on the effectiveness of biopesticides because in general, the use of chemicals shows a faster effect than natural methods. In addition, the price of organic products in the market is also expensive and burdensome for farmers. Through studies like these, farmers have the potential to use inexpensive materials to make their own biopesticides for application on their respective farms.

Despite many studies on plant extract, research on kaffir lime leaf extract as biopesticide is scarce, especially on chilli. Several studies reported the kaffir lime extract with its ability as insect's repellent (Ikawati *et al.,* 2017; Lim and Majid, 2019; Nawi *et al.,* 2020). In 2010, Noveriza conducted a study on the effect of bay leaf and kaffir lime leaf extracts on *Fusarium oxysporum*. They found that 5% of methanol extract of Kaffir lime leaf significantly inhibited the growth of *F. oxysporum* with 95.6% of inhibition. Based on previous studies, it can be summarised that kaffir lime leaves have a high potential as biopesticides. Since they are abundantly found in Malaysia, therefore, this study was conducted to evaluate

the effect of kaffir lime leaves extracts on pre and postharvest performance of chilli plants.

MATERIALS AND METHODS

Study Plot and Planting Materials

This study was established at Rhu Tapai Centre of Agriculture, Setiu, Terengganu, Malaysia (5°30'48.6"N, 102°58'38.5"E). The planting period was from June to October 2019. In this study, the *Capsicum annuum* (chilli) Hybrid-461 was used and the seedlings were planted within 1 x 1 m planting distance in the experiment plots by using an open fertigation system. All chilli plants were applied with the same amount of fertilizer and similar handling activities based on standard agricultural practice to ensure that all plants were growing healthily.

Preparation and Application of Kaffir Lime Extract

The plant extract was obtained from kaffir lime leaves. The leaves were picked randomly from the tree and taken to the laboratory at the University Malaysia Terengganu. All leaves were washed under running tap water and left under the shade to air dry for two days. Then, a total of 200 g of dried fresh kaffir lime leaves were mixed with 1L of deionized water and homogenized in a Panasonic MXSM1031 mixer, the mixture was filtered through muslin cloth and the leaf extract was left in the refrigerator for 24 hours. It was diluted into 25%, 50% and 75% (v/v) to a final volume of 1L (Subramaniam *et al.,* 2005). The solution was used to spray all the chilli plants from bottom to top with an average dose of 50 ml for each plant. The solutions were applied to the chilli plants every two weeks from the first two weeks after planting until chilli was harvested. For chemical pesticides, the Zesban EC Chlorpyrifos 21.2% brand used by farmers in Malaysia was applied with 2.4 ml of chemical pesticides, diluted in 1L with deionized water and sprayed on the entire part of the chilli plant at an average dose of 50 ml per plant.

Experimental Design

Prepared leaf extracts and commercial chemical insecticides were applied according to the experimental design (Table 01). Study plots were allocated using a Randomized Complete Block Design (RCBD) with a total of 10 replicates. The randomization was performed using the table of random numbers (Gomez and Gomez, 1984).

Data Collection

Preharvest parameters

Plant height: The height of the plants was measured in centimetre (cm) from 1 cm above soil line to shoot tip on the field at two weeks' interval by using a measuring tape.

Stem diameter: The diameter of the chilli plants was measured every two weeks starting from the second week after planting by using a digital Vernier calliper and expressed in units of millimetre (mm).

Table 01: Treatments used in the study

Treatment	Description
P1	No pesticide applied
P2	Chemical pesticide
P3	25% kaffir lime extract
P4	50% kaffir lime extract
P5	75% kaffir lime extract

Table 02: The pest severity score

Score	Percentage of leave damage caused by pest infestation (%)	Level
0	0	No pest infestation
1	1-25	Scattered appearance of few pests on the plant
2	26-50	Severe infestation of pests on any one branch of the plant.
3	51-75	Severe infestation of pests on more than one branch or half portion of the plant
4	76-100	Severe infestation of pest on the whole plant

Crown diameter: The sum of two values taken in two perpendicular axes from the plant's top and expressed in centimetres (cm).

Pest severity: The data were recorded every two weeks from the second week after planting. As noted in Table 02, the modification of pest severity scoring was based on percentage of the pest infestation (Ruchika and Kumar, 2012).

Postharvest Parameter

Fresh weight of chilli: The fresh weight was recorded at harvesting stages and the former was expressed in kilogram (kg).

Leaf area: The leaf area was measured by using the leaf area meter (Model CI-202, CID Bio--Science Inc., Camas, WA) after harvest and expressed in unit of cm^2.

Chilli Colorimetry: Colour indices were recorded with a Minolta Chroma Meter (Model R200 Trimulus Colour Analyzer, Minolta Camera Co. Ltd., Japan). The instrument was set to the white standard for calibration. Colour was measured on three sides of the chilli. The data were expressed as L*, a*, and b* values. L* represents the luminance factor going from 0 (black) to 100 (white). a* ranges from -60 to +60, indicating the colour red (+60) and green (-60). During this time, b* ranges from -60 to +60, indicating yellow (+60) and blue (-60). a* and b * were then used to calculate the hue angle (h° = tan1 b*/ a*) for colour interpretation. The hue angle (h°) represents purple-red (0°), yellow (90°), bluish-green (180°), and blue (270°). Chroma (C*) is the intensity or saturation of a colour where a low value indicates a dull colour while a high value indicates a vivid colour, where it is calculated from (a * 2 b * 2) (McGuire, 1992). For each treatment, ten samples were measured separately and the mean of ten measurements was calculated.

Firmness and soluble solid concentration (SSC): The chilli was cut into 2 x 2 cm pieces. Chilli firmness was measured using TA-XT2 plus Texture Analyzer (Stable Micro System, Godalming, UK) with a 2 mm stainless steel probe (P2) and the data were recorded in a unit of

Newton (N). Meanwhile, SSC was measured using digital refractometer and expressed in °Brix.

Statistical Analysis

Data were analysed using One-way ANOVA. The statistical analysis was performed using IBM SPSS statistical software version 20.0, IBM Corp, Armonk, USA.

RESULTS AND DISCUSSION

Preharvest parameter

Application of kaffir lime leaf extract on chilli plants had a positive impact mainly on pest severity and plant height attributes. Sprayed the chilli plants with kaffir lime leaf extract ranged of 25, 50 and 75% (v/v) that had successfully reduced the pest severity on the plants (Table 03). In fact, kaffir lime extract had significantly ($P<0.05$) suppressed the pest severity when

compared to chemical pesticides. According to Loh *et al.* (2011), essential oil of kaffir lime leaves was effectively killing the larvae of tobacco army worm, *Spodoptera litura,* which is one of the common pest in vegetables farm. They found that the essential oil had antifeedant properties due to β-citronellol (6.59%), linalool (3.90%) and citronellol (1.76%) content which resulted in severe growth inhibition of *S. litura*. Although in this study, the kaffir lime leaf extract compounds were not evaluated, the leaf extract might have similar insecticidal properties of the essential oil. On the other hand, the kaffir lime leaf extracts' effect was more superior than the chemical pesticides which could be due to the resistance developed in the insect pest towards chemical pesticides in the planting area.

Lack of studies shows the potential of kaffir lime extract on pest severity. However, kaffir lime extract is known to have an antimicrobial effect such as antibacterial and antifungal properties (Lanciotti *et al.,* 2004). The methanolic extract from kaffir lime' leaves can inhibit herpes virus and can be used as mosquito repellent (Chowdhury *et al.,* 2009). In addition, several studies reported that the kaffir lime extract shows its ability as an insect repellent (Ikawati *et al.,* 2017; Lim and Majid, 2019; Nawi *et al.,* 2020). In a study conducted by Noveriza (2010), 5% of methanol extract of bay leaf and kaffir lime extracts can significantly inhibit (95.6%) the *F. oxysporum*. The results of this study showed higher pest severity and infestation in the chilli plants treated with chemical pesticides. It was suggested that the insects' pests surrounding the planting area have built-up the resistance against the chemical pesticides. Achio *et al.* (2012) reported that frequent use of chemical fungicides can increase the resistance of insect pests in the planting area. Several studies reported the kaffir lime extract with its ability as an insect repellent. It also might be due to the extract's active ingredients to create a vapour barrier with a repulsive flavour or smell to insects, preventing them from coming into touch with the treated regions (Nerio *et al.,* 2010). Plant extracts and essential oils also have larvicidal and ovicidal effects on insects, decrease oviposition, decrease respiration, diminish adult emergence, and make it difficult to identify host plants (Ali *et al.,* 2017). They also serve as antifeedants, repellents, and attractants. It is also supported by previous study that the leaf, peel, and fruit of the kaffir lime, in particular, have therapeutic qualities (Srisukh *et al.* 2012) and insect repellent action (Tawatsin *et al.* 2001).

Height measurement is an important attribute in measuring crop plant growth as it influences crop architecture, apical dominance, biomass, resistance to lodging, tolerance to crowding and mechanical harvesting (Liu *et al.,* 2018). In this study, application of 50% kaffir lime leaf extract treatment had significantly produced taller plants if compared to chemical pesticides. It is notable that application of kaffir lime leaf extract had reduced the pest severity. Hence, plants sprayed with the kaffir lime leaf extracts found to be healthier that promoted a taller plant growth.

Table 03: Effect of different treatments on the preharvest parameter

Treatments	Attributes			
	Pest severity	Plant height (cm)	Stem diameter (mm)	Crown diameter (cm)
No Pesticide	2.02[b]	27.64ab	6.53	29.07
Chemical	2.38[b]	25.28[b]	6.56	27.39
25% kaffir lime extract	1.05[a]	28.44ab	7.84	28.36
50% kaffir lime extract	1.41[a]	29.31[a]	6.94	26.92
75% kaffir lime extract	1.38[a]	27.61ab	7.62	27.54
p- value	0.000**	0.040**	ns	ns

*Mean values with the same letter in the same column for each attribute are not significantly different at p<0.05, **: Significant at p<0.05, ns = not significant.*

Results presented in the table are average results from two weeks after planting until harvesting.

The previous study has shown a very strong relationship between plant extract and plant growth. In study conducted by Alam *et al.* (2014), the application of seaweed extract has increased the root yield of carrot due to the increment of soil microbial. Plant extracts have been used for decades as foliar- and soil-applied treatments in crop production systems due to the presence of a number of plant growth-stimulating compounds (Wally *et al.,* 2012). Not only that, the plant extract is biodegradable, non-toxic, non-polluting and non-hazardous to humans, and animals. For that reason, it could help to provide a favourable condition for more abundant microbial community. Thus, the plants are able to take up the nutrient more efficiently and promote better growth.

Postharvest Parameters

Different concentrations of kaffir lime leaf extract sprayed on the chilli plants had significant effects on chilli fresh weight and number of chilli pods (Table 04). The chilli collected from the plants sprayed with 25% kaffir lime leaf extract was 1.17 kg heavier than the chilli obtained from the control plants. However, treated the plants with either chemical pesticides or different concentrations of kaffir lime leaf extract had produced similar chilli fresh weight which was better than the control. This indicates that pest control is critically required in chilli plants regardless the type of the pest control used. Results from this study is aligned with Awang *et al.* (2015) showing that treated chilli plants with pesticides had a better growth performance and yield if compared to untreated plants.

Results present in Table 04 indicates that fresh weight of chilli is directly related to the number of chilli pods. Hence sprayed the plants with 25% of kaffir lime leaf extract had the highest number of chilli pods, followed by 75% kaffir lime leaf extract, chemical pesticides, 50% kaffir lime leaf extract and control. However, kaffir lime leaf extract had no significant effect on plant leaf area, chilli pod total soluble solid content and the firmness. Finding from this study agrees with the previous study which reported that the application of plant extract such as Moringa extract in the form of foliar spray has significantly increased the yield of onions, bell pepper, soya beans, sorghum, coffee, tea, chili, melon, maize (Fuglie, 2000), and tomato (Culver *et al.,* 2012).

Colour is the primary consistency characteristic that affects the market produce selection (Malakar *et al.,* 2018). During harvesting times, lightness (L*), chroma (C*), and hue angle (h°) of capsicum fruits were measured to monitor surface colour changes. Colour is an important factor in determining consistency and end-user acceptance, including chillies. In red chillies, the red lycopene pigment is degraded while anthocyanin is synthesized concurrently during ripening (Aza-González *et al.,* 2017). According to Naimah *et al.* (2016), higher value of a* while lower value of b*, L*, h° and C* showed that the chilli sample has more intense colour of red. Statistical analysis for colour values of chilli samples is shows in Table 05. In this study, various biopesticides' application did not significantly affect the colour indices of chilli below.

Table 04: Effect of different treatment on the postharvest parameter

Treatments	Fresh weight of chilli per plant (kg)	No. of chilli pods per plant	Soluble solid concentration (°Brix)	Firmness of fruit (N)	Leaf area (cm2)
No Pesticide	3.39^{b}	690^{b}	7.53	6.31	750.07
Chemical	4.45^{ab}	806^{ab}	7.40	7.40	813.81
25% kaffir lime extract	5.10^{a}	991^{a}	7.53	9.61	892.50
50% kaffir lime extract	4.31^{ab}	753^{ab}	6.43	6.25	793.40
75% kaffir lime extract	4.42^{ab}	837^{ab}	7.47	6.30	760.09
p- value	0.024**	0.008**	ns	ns	ns

*Mean values with the same letter in the same column for each attribute are not significantly different at p<0.05, **: Significant at p<0.05, ns = not significant.*

Table 05: Effect of kaffir lime extracts on lightness (L*), chromaticity value a*, b*, hue angle (h°), and chroma (C*) of chilli

Treatments	Attributes				
	L^*	a^*	b^*	h°	C^*
No Pesticide	38.19	36.98	22.06	30.83	43.07
Chemical	38.59	36.68	22.36	31.36	42.96
25% kaffir lime extract	37.97	34.34	23.81	34.65	41.81
50% kaffir lime extract	38.20	36.19	22.63	31.90	42.69
75% kaffir lime extract	37.52	36.62	23.00	32.04	43.27

*Mean values with the same letter in the same column for each attribute are not significantly different at $p<0.05$, *: Significant at $p<0.05$, ns = not significant.*

CONCLUSIONS

Finding an alternative biopesticide towards chemical pesticides is an effort in ensuring the longevity of human and the earth, especially on chilly plants that is well known for its susceptibility for pest infestation and disease infection. This study on the effect of kaffir lime leaf extract on the chilli plants had produced some positive results that were able to produce healthier chilli plants with higher yield. This study also revealed that 25% of kaffir lime leaf extract had a potential as alternative biopesticides for chilli plants as it produced the tallest plants and the highest number of yield from a chilli pod. This procedure is suggested to be used widely by Malaysian farmers in order to mitigate frequent application of chemical pesticides.

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