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EFFECT OF REFUGIA PLANTS ON WHITEFLY POPULATION AND RED CHILI (*CAPSICUM ANNUUM* L.) PRODUCTION

JASMI*, M. AFRILLAH, and A. NASUTION

Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University, Meulaboh, Indonesia
*Corresponding author's email: Jasmi@utu.ac.id
Email addresses of co-authors: muhammadafrillah@utu.ac.id, aswinnasution@utu.ac.id

SUMMARY

Determining the effects of the refugia plant on the whitefly population and the production of several cultivars of red chili (*Capsicum annuum* L.) was the focus of research conducted from March to June 2019 in the experimental garden, Faculty of Agriculture, Teuku Umar University, Meulaboh, West Aceh Regency, Indonesia. The genetic materials consisted of three chili genotypes, i.e., cultivar TM-999 and two hybrid cultivars, Lado F_1 and Lidia F_1. The experiment in a 3 × 2 split plot design had three replications. The factor studied was the presence of a whitefly. The Refugia (R) plants, used as main plots, included R0 = Control, R1 = Zinnia, and R2 = Kenikir. Red chili genotypes comprised the sub-plots, i.e., V1 = TM-999, V2 = Lado F_1, and V3 = Lidia F_1. The studied variables were the whitefly population determination, the percentage of attack rate, and the chili production per plot. The results showed that Refugia plants greatly affected the whitefly population, portion of pest attack rates, and chili production per plot. Specifically, the refugia plant highly affected the whitefly population (Refugia kenikir 1.41% compared with control 11.89%), the percentage rate of pest attack (Refugia kenikir 44.44% compared with 100% of check), and production per plot (kenikir 23.59 g compared with the control's 23.07 g). Refugia plants are influential in reducing whitefly development/production.

Keywords: red chili (*Capsicum annuum* L.), chili production, Refugia plants, whitefly population

Key findings: Refugia developed a microhabitat that expects to contribute to efforts to conserve natural enemies. Refugia plants can be effective pest traps, especially whiteflies on red chili plants, because, in addition to providing temporary shelter for natural enemies of pests, refugia also benefits from biotic ecosystem interactions.

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INTRODUCTION

Refugia are several types of plants that can provide shelter, food sources, and other resources for natural enemies, such as, predators and parasitoids. It also functions as a microhabitat, providing a suitable environment to conserve natural enemies (Aqilah, 2016). Refugia plants can serve as pest traps, especially for whiteflies on red chili plants. In addition to providing provisional shelter for natural enemies of pests, refugia also gives settlement through interactions with biotic ecosystems (Martono, 2015).

Whitefly is an eminent pest species that harms chili farms a lot. Whiteflies, also known to act as paramount vectors of yellow virus disease, transmit the virus to plants. Red chili (*Capsicum annuum* L.) is one of the most valuable vegetables in Indonesia and worldwide (Amams *et al*., 2023). Besides having high nutritional value, chili has a high economic value (Maulani *et al*., 2023). In Indonesia, the demand for chilies has an upward trend yearly; however, its production has declined due to pests, especially the whitefly. Whitefly symptoms can be visible in necrotic spots and chlorosis on leaves caused by leaf tissues due to nymphs and adult attacks. In extreme whitefly population conditions, it can also inhibit and retard the plant growth. By sucking the plant sap, it damages the chili plants more, resulting in leaf chlorosis and falling, reducing the growth and yield of chilies. Indirect damage is the honeydew released by pests, which can cause black sooty fungus attacks at various plant stages, severely affecting and reducing plant photosynthesis (Lin *et al.,* 2005; Daryanto *et al*., 2021).

Farmers mostly try to control whiteflies conventionally through insecticides. However, such chemical control has unsatisfactory results because the whitefly quickly becomes resistant to new insecticides, increasing production costs. The chemicals controlling these insects also harm the environment, including the carnage of existing biological agents (Azmi and Leksono, 2014). Therefore, environmentally friendly strategies require planning to control the said pest.

Utilization of refugia plants has the potential as a biological control agent in cultivated plants. Refugia plants provide transitory shelter to natural enemies of the pests, such as, predators and parasitoids, as well as, supporting components of biotic interactions in ecosystems, i.e., pollinating insects (Pracaya, 2008). These types of refugia plants can serve as an alternative for controlling whitefly infestation in sunflowers, peppers, marigolds, setaria grass, spinach (Azmi and Leksono, 2014), corn, and long bean plants (Setyadin *et al.,* 2017).

Refugia plants have flowers that are brightly colored and contain essential oils, which can generally repel pests (Mahmud and Taufid, 2006). Kenikir plant flowers contain saponins, flavonoids, polyphenols, and essential oils. Bougainvillea has various colors and flowers that always bloom so that they interact well with natural enemies (Setyadin *et al.,* 2017). These factors lead to the importance of controlling whiteflies on chili plants by planting refugia as a chili hedge.

According to the general classification of Sudiono and Purnomo (2009), the whitefly belongs to the kingdom - Metazoa, phylum - Arthropoda, class - Insects, order - Hemiptera, suborder - Sternorrhyncha, superfamily - Aleyrodidae, genus - *Bemisia*, and species - *Bemisia tabaci.* A whitefly goes through metamorphosis consisting of three phases, i.e., egg, nymph, and imago (Figure 1). Eggs are oval, slightly curved like a banana fruit, bright yellow, between 0.2–0.3 mm long. On average, virus-infected leaves and healthy leaves, the eggs were 77 and 14, respectively. The average egg stage length is five to eight days (Hidayat, 2004).

Nymphs consist of three instars. The first instar is oval and flat, greenish-yellow in color, and has limbs that function to move. Nymphs of the 2nd and 3rd instars are sessile and will stick to the leaves during their growth period. The nymphal stage averages 9.2 days (Hendrival, 2010). Imago is an adult insect whose body is small, between 1–1.5 mm, white, and its wings are transparent, covered with a layer of starchy wax (Hendrival, 2010).

The first symptoms of a whitefly attack can be visible with yellow spots on young leaves;

Figure 1. Whitefly eggs, nymphs, and imago.

Figure 2. Development of yellow virus symptoms in chili plants caused by whiteflies.

eventually, the leaf veins turn yellowish, sunken, wrinkled, smaller, and thicker on chili plants. Furthermore, the leaf buds will become completely yellow or mixed with green. This attack disrupts the process of photosynthesis, resulting in stunting of plants (Hidayat, 2004; Poudyal *et al*., 2023).

The whitefly attacks the chili plants during vegetative and generative phases. The whitefly sucks sap from the chili plant leaves in the nursery when the chili plants start to grow, till the leaves turn yellow, and until harvest. The whitefly pest phases from eggs to nymphs to imago attack the chili plants. Whitefly imago likes young leaves on top of plants, and its attack can cause economic losses because the yellow virus secreted by the whitefly can inhibit the growth of chili plants (Figure 2); eventually, the chilies are unharvestable (Meilin, 2014). The presented study determined the effects of refugia plants on whitefly populations and the production traits of several cultivars of red chili (*Capsicum annuum* L.).

MATERIALS AND METHODS

This research ran from March to June 2019 at the experimental garden of the Faculty of Agriculture, University of Teuku Umar, West Aceh District, Indonesia. The genetic material used included three chili genotypes, i.e., cultivar TM-999 and F_1 hybrids, Lado and Lidia. The experiment in a 3 × 2 split plot design had three replications. The NPK fertilizer and manure applications were at the recommended doses. The main plots comprised Refugia (R) with three levels, namely, R0 = Control, R1 = Zinnia plants, and R2 = Kenikir plants. The sub-plots were the chili genotypes, i.e., V1 = TM 999, V2 = F_1 hybrid Lado, and V3 = F_1 hybrid Lidia. The recommended cultural practices and irrigation also maintained the chili crop.

Preparing the seeds of zinnia and kenikir began with sowing these in polybags measuring 40 cm × 50 cm containing 10 kg of sandy loam soil. Refugia seedlings' transfer to the field ensued at the age of ±1 month, with the chili seedbed preparation completed. Planting the refugia plants around a patch of chili plants resembled a fence. Plant maintenance included watering every day in the morning and evening if without rain, and weeding around the polybag occurred every two weeks. The NPK fertilizer (16:16:16) application continued by dissolving 10 grams of NPK in one liter of water. Chili harvesting depended on the genotypes where the F_1 hybrid Lidia was at 85 days after planting (DAP), while the cultivar TM 999 at 100 DAP, and F_1 hybrid Lado at 90 DAP at fruit maturity.

Observations

Whitefly population

Counting three sample plants in each treatment per replication tallied the whitefly population. Observations happened six times in the growing season at the age of 15, 30, 45, 60, 75, and 85 DAP. The abundance of whitefly population calculation was in the imago phase using the relative abundance formula as below:

$$Ki = ni/N \times 100$$

Where:
Ki = population abundance
Ni = number of individuals of the i^{-th} species
N = total number of individuals of all species
Percentage level of pest attack

Percentage rate of whitefly

On chili plants, the whitefly attack began from the seedbed and took place until harvest. The percentage rate of whitefly infestation calculation was as follows:

$$P = a/b \times 100$$

Where:
P = Percentage level of attack (%)
a = Number of affected plants per plot
b = Total number of plants

Chili yield per plot (g)

The chili production and fruit yield measurement was according to the harvesting age of each genotype by weighing its fruit production in each plot.

Data analysis

The ANOVA test could only indicate no difference between the averages of all treatments. It may not provide information if there is any difference between an individual treatment with another separate treatment. Simply put, if there are five treatments for testing, for example, treatments A, B, C, D, and E, if the ANOVA test shows a significant difference, it can be a conclusion for an overall significant difference between the average treatments, but not for the average treatment A having a difference from the average treatment B, and so on.

For more in-depth tests, it is necessary to carry out further trials (Post hoc tests). There are various advanced tests, but this article chose the BNT test. The BNT test, commonly known as the LSD (the least significant difference) analysis, is a method introduced by Ronald Fisher. This method uses the BNT or LSD value as a reference to determine whether the average of the two treatments is statistically different or not. In calculating the BNT or LSD value, some data need to come from the calculation of variance (ANOVA) that has resulted before. The data is in MSE and dfE and needs a t-student table. The complete formula is as follows:

$$\text{BNT } 0.05 = \text{t0.05 (dbg)}\sqrt{\frac{2Kt\,g}{r}}$$

Where:
BNT 0.05 = The smallest significant difference at the 5% level
t0.05 (dbg) = Standard value of q at 5% level
Ktg = Middle square of error
u = number of replications

RESULTS AND DISCUSSION

Whitefly population

The average whitefly populations at 15, 30, 45, 60, and 75 DAP (days after planting) of chili plants against refugia plants after testing with BNT 0.05 is in Table 1. The results revealed that the largest population of whitefly infestation surfaced in the control treatment, while the small population was in the treatment of the refugia plant kenikir (Table 1). It authenticated that the use of refugia plants has a significant impact on reducing the whitefly population on chili plants. Refugia plants lodged around chili plants can act as pest control plants and temporarily shelter their natural enemies. These results follow the findings that refugia plants provide one of the temporary shelters that can meet the needs of insect natural enemies (Pujiastuti *et al*., 2015). Hadi (2009) also explained that insects and plants have a reciprocal relationship, in which both will always benefit.

Although, in the two types of refugia plants (zinnia and kenikir), the whitefly population was not significantly different. However, by comparison, and at all ages of observation, the whitefly population on chili plants treated with refugia kenikir (R2) was less than in the treatment of refugia zinnia (R1). It might be due to the different flower colors' influence on the two types of refugia plants. The zinnia flower color was pink, while the kenikir flower was yellow. According to Natawigena (1990), various kinds of insects have a positive relationship and more attraction for color, and the yellow flower was more attractive than other colors.

The results showed that whitefly populations were lower on chili plants treated with the kenikir refugia (R2) than those treated with the refugia zinnia (R1), maybe due to the influences of the different flower colors in the two treatments. Treatment refugia zinnia (R1) has the flower colored pink and refugia kenikir (R2) flower colored yellow.

Table 1. Average whitefly population at 15, 30, 45, 60, and 75 DAP on chili plants with two types of refugia plants.

Treatments	Whitefly population (#)				
	15 days after planting	30 days after planting	45 days after planting	60 days after planting	75 days after planting
Control (R_0)	7.67 b	8.89 b	14.44 b	22.11 b	23.07 b
Zinnia (R_1)	0.93 a	0.85 a	1.26 a	1.11 a	1.41 a
Kenikir (R_2)	0.19 a	0.22 a	0.93 a	1.04 a	1.07 a
BNT $_{0.05}$	0.83	0.96	1.46	2.29	1.87

Note: Numbers followed by the same letter on the same line that are different are not significant in the 0.05% BNT test.

Insects' attraction to yellow color also has manipulations by the wavelength of light. According to Marikun M *et al*. (2014), insects can receive light of more wavelength than humans (ranging from 540 to 600 nm), and insects prefer yellow because of a higher wavelength than other colors. According to Hasibuan (2018), the yellow color has a wavelength of 560–590 nm, the red color ranges from 630 to 700 nm, and green from 480 to 560 nm, and creatures can receive the color with a wavelength ranging from 560 to 590 nm. Adawiyah *et al.* (2020) suggested that pink flowers can attract insects, such as, ladybugs and wasps, yellow flowers attract ladybugs, army flies, and butterflies, orange attract koksi beetles and butterflies, while white attract flower flies, koksi beetles, fruit flies, and ants.

With the presence of refugia plants, the insect pest population and herbivores can significantly decrease on cultivated plants. In this study, the refugia plant served as a natural enemy plant, especially the natural enemy of whitefly. The presence of natural enemies can reduce the whitefly population on the chili plants. Pujiastuti *et al.* (2015) findings showed rice plants surrounded by refugia plants received fewer herbivorous insects than rice fields without refugia plants during the vegetative and generative phases.

The refugia plants had a significant effect on the whitefly population (1.41% kenikir refugia compared with 11.89% control), the percentage of pest attacks (44.44% kenikir refugia compared with 100% control), and chili fruit production (23.59 g kenikir compared with control 23.07 g). Hence, refugia plants can reduce whitefly attacks versus those without refugia (control).

The average whitefly population at the 15, 30, 45, 60, and 75 DAP on the plants of three chili cultivars is available in Table 4. The whitefly (*Bemisia tabaci)* population on chili plants of all the cultivars did not show significant differences, and the three chili genotypes have the same resistance to whitefly. Plant resistance arises naturally, possibly caused by fluctuating biological, chemical, and environmental conditions related to plant growth. Study results agree with the opinion of Sodiq (2009) that plants have some natural resistance to insect pests, which makes plants active in rejecting, preventing, and tolerating pest attacks that arise due to the intervention of physical, chemical, genetic, and environmental factors.

Previous studies have shown that waxy plants, i.e., crucifers, are less favorable to *Bemisia tabaci* (Heather, 2002). Whiteflies dislike plants with lots of hair (Bedford *et al.*, 1994), whereas, according to Heather (2002), they prefer soft leaves less. The research results of Setiawati *et al*. (2008) stated that the red chili that *Bemisia tabaci* liked the most was the taro variety, and the most disliked was the hot chili variety. It is due to several factors, including leaf thickness, leaf hair density, sugar content in the trichome glands, protein content found in plants, chemical constituents, such as, a-tocopherol, squalene, and linolenic acid, and secondary metabolites, such as, solanine, solasodine, tomatidine, and tomatin. Hirano *et al*. (1993) reported that host quality also greatly influenced the population abundance of *B. tabaci*. The physical characteristics that affect the attractiveness of *B. tabaci* are leaf hairs, thickness, and shape, while the chemical characteristics are pH and leaf sap.

Pest attack rate

The average percentage of pest attack rates on chili plants against several types of refugia plants after being tested with a BNT of 0.05 appears in Table 2. The results revealed that the highest percentage of pest attack rates emerged in the control treatment, whereas the lowest attack was in the refugia plant kenikir. The low level of pest attack on chili plants with refugia treatments, kenikir, and zinnia, versus the control, is assumable because refugia plants can function as alternative hosts; thus, the level of pest attack on chili plants was low. According to Altieri and Nicholls (2004) and Sepe and Djafar (2018), refugia plants are vital components of agroecosystems because they can positively influence the biology and dynamics of natural enemies. Refugia plants planted around the plantations functioned as shelter and refuge for natural enemies when environmental conditions were not suitable and also provided alternative hosts and additional food for parasitoid imagos, such as, pollens, nectars, and honeydew in flowering plants.

Each type of pest has a natural control from a complex of natural enemies, which include predators, parasitoids, and pest pathogens. Compared with pesticide use, employing natural enemies is organic, effective, inexpensive, and harmless to health and the environment (Untung, 2006). If the natural enemies can act as predators optimally from the start, pest populations' level of fluctuation with natural enemies gains a balance, preventing pest explosion (Maredia *et al.*, 2003). The smaller the pest population, the smaller the attack percentage (Tables 1 and 2).

The average percentage of pest attack rates on the plants of three chili cultivars after being tested with a BNT of 0.05 occurs in Table 5. The highest percentage of pest attack rates on chili plants resulted in the F_1 hybrid Lado, significantly different from the cultivar TM-999 (V1) but not relevantly different from the F_1 hybrid Lidia. It might be due to differences in the resistance genes found in the three chili cultivars and the plant morphology, and essential amino acids and other chemical substances found in the leaves that affect the level of pest attack on the chili plants. Berlinger (1986) stated that the physical characteristics that affect the attractiveness of whiteflies are leaf hairs, thickness, and shape, while chemical attributes are pH and leaf sap.

Red chili production

Table 3 presents the average fruit production per plot of the three chili genotype plants with two types of refugia plants (zinnia and kenikir) after being tested with a BNT of 0.05. The production per plot of chili plants in the treatment of refugia kenikir was significantly different from the control treatment but not notably different from the treatment of refugia zinnia plants. The yield increase in chili plants with the dose of refugia kenikir and zinnia plants may refer to less attack by whitefly pests. According to Lisdayani *et al.* (2017), the use of non-host plants in crop cultivation systems, apart from acting as a disguise that makes host plants hard to find, also acts as a physical barrier for pests to find the cultivated plants.

The lowest fruit production came from the control treatment because the high whitefly attack reduced the chili fruit production. The low yield of chili fruit, apart from the small amount produced, was also the relatively small size of the fruit. Whitefly pests attack chili plants when the plants are still young (vegetative), thus affecting the growth and flowering phase of chili plants. The population density of the whitefly causes damage to the leaves of the chili plants; as a result, the chili plants cannot carry out the photosynthesis process properly. Whiteflies damage plant leaves because they pierce the plant tissue and suck the leaf cell sap, which causes the leaves to grow abnormally and the affected parts of the leaves to become brittle (Gardner, 1991; Anggraini *et al.*, 2018).

Leaf damage by pests also affects the growth phase of the plants. In the vegetative stage until the beginning of fruit formation, all photosynthetic activities contribute to stems and leaves for plant growth. In the early phase of fruit formation, photosynthetic generations are temporarily stored in the stem and then transferred to the fruits. If there is injury to the leaves, the photosynthesis substances, earlier sent to the fruit, are reabsorbed to form new leaves as compensation for the damage. When in the fruit formation phase, temporary storage of photosynthetic products in the stems did not transpire, then from the dynamics of the photosynthetic results, the critical level for leaf damage is fruit formation. In this phase, damage to the leaves will cause reduced photosynthetic substances sent to the fruit, thereby a lesser fruit amount produced, resulting in low plant production (Hendrival *et al.,* 2013).

The average fruit production of three chili cultivars after being tested with a BNT of 0.05 appears in Table 6. The increased fruit production of chili cultivar TM-999 (V1) and F_1 hybrid Lidia was due to their tolerance to whiteflies. According to Sodiq (2009**),** with resilience, the cultivars can heal themselves from damage by pests, even though the pest

Table 2. The average percentage of pest attack rates on chili plants with two types of refugia plants.

Treatments	Pest attack (%)
Control (R_0)	100.00 b
Zinnia (R_1)	44.44 a
Kenikir (R_2)	29.63 a
BNT $_{0.05}$	16.36

Note: Numbers followed by the same letter on the same line that are different are not significant in the 0.05% BNT test.

Table 3. Average fruit production per plot of chili plants with two types of refugia plants.

Treatments	Fruit production plot^{-1} (g)
Control (R_0)	11.89 a
Zinnia (R_1)	23.59 b
Kenikir (R_2)	26.98 b
BNT $_{0.05}$	3.46

Note: Numbers followed by the same letter on the same line that are different are not significant in the 0.05% BNT test.

Table 4.The average population of whitefly at 15, 30, 45, 60, and 75 DAP on three chili cultivars.

Treatments	Whitefly population				
	15 days after planting	30 days after planting	45 days after planting	60 days after planting	75 days after planting
TM 999(V_1)	2.33	3.22	4.96	7.41	8.56
Lado F_1 (V_2)	3.30	3.26	6.41	9.15	9.52
Lidia F_1 (V_3)	3.15	3.48	5.26	7.70	7.48

Table 5. The average percentage of pest attack rates on three chili cultivars.

Treatments	Pest attack (%)
TM 999(V_1)	48.15 a
Lado F_1 (V_2)	74.07 b
Lidia F_1 (V_3)	66.67 ab
BNT $_{0.05}$	20.31

Note: Numbers followed by the same letter on the same line that are different are not significant in the 0.05% BNT test.

Table 6. Average fruit production per plot of three chili cultivars.

Treatments	Fruit production plot^{-1} (g)
TM 999(V_1)	23.16 b
Lado F_1 (V_2)	18.66 a
Lidia F_1 (V_3)	20.15 ab
BNT $_{0.05}$	3.46

Note: Numbers followed by the same letter on the same line that are different are not significant in the 0.05% BNT test.

attack is equal to the sensitive genotypes. The chewing mouth-type insect attacks plants by eating the plant parts. Therefore, the only type of tolerance that can result is replacement. This regrowth has amelioration, often from the relative maturity level at which the damage to plant parts occurred.

Refugia plants that can generally be beneficial have flowers that are brightly colored and contain essential oils that can repel pests, such as, kenikir flowers containing saponins, flavonoids, polyphenols, and essential oils (Mahmud and Taufid, 2006). Bougainvillea has various colors and flowers that always bloom so that they interact well with natural enemies (Setyadin *et al*., 2017). Types of plants that have the potential to be effective as refugia plants are flowering plants that have striking colors, such as, sunflowers, paper flowers, cock's comb flowers (Celosia), kenikir flowers, and chicken droppings flowers (marigold).

CONCLUSIONS

The refugia plants significantly affected the whitefly population (1.41% in kenikir refugia compared with 11.89% of the control), the percentage of pest attacks (44.44% in kenikir refugia compared with 100% of the check), and chili fruit production (23.59 g kenikir compared with the control's 23.07 g). Hence, refugia plants can reduce whitefly attacks compared to those without them (control).

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