# Shadecovers Affect Degradation of Carbaryl on Field-grown Pakchoi

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**Additional index words.** ***Brassica rapa*** **subsp. *chinensis*, enzyme-linked immunosorbent assay, ELISA, pesticide residue**

**Summary. The effect of shade covers on degradation of insecticide, carbaryl on field-grown pakchoi (*Brassica rapa* subsp. *chinensis*) was examined by a commercial enzyme-linked immunosorbent assay (ELISA) kit. Carbaryl at a.i. 10.6 g·L$^{-1}$ (1.42 oz/gal) was applied to the plants grown under five different shade treatments including control without any coverings. The experiment was arranged in a randomized complete block design with three replications. Pesticide residue on leaf tissues was examined on dates of 1, 3, 5, and 7 days after pesticide application. On all sampling dates, pesticide residue was greater with treatments with higher shade percentage. Both linear and quadratic relationship of shade (independent variable) and the concentration of remained carbaryl (dependent variable) were significant ($P < 0.05$). The half-life of carbaryl on pakchoi leaves ranged from 2 days for control to 9 days for the heaviest shade (75%) treatment with rain protection.**

Degradation of pesticides on field-grown crops varies depending on agro-climatic conditions (Mann and Chopra, 1969; Yadav and Jagalan, 1982). Such environmental factors include the amount of sunlight, air temperature, relative humidity, wind speed and precipitation (Crosby, 1969; Demeterio, 1983; Mitchell, 1961; Willis et al., 1996, 1988). The half-life of carbaryl was reported elsewhere ranging from nearly 1 d to more than 1 month depending on the type of crops and growing conditions. In temperate regions the half-life of carbaryl on eggplant (*Solanum melongena*) was 6.5 d (Deshmukh and Lal, 1970) and only 1 d in an area with tropic climate in India (Mann and Chopra, 1969). On citrus grown in the temperate region the half-life of carbaryl was 28 d on lemons (*Citrus limon*) and 42 d on oranges (*Citrus sinensis*) (Gunther et al., 1962). On peaches (*Prunus persica*), apples (*Malus* spp.), strawberries (*Fragaria* spp.) and gooseberries (*Ribes grossularioides*) it ranged from 7 to 10 d (Bogomolova, 1968; Polize et al., 1971).

Various types of polypropylene materials can be used as shade covers to protect growing leafy vegetables from high light intensity and high temperature in the tropics. The degree of shading may influence the persistence of a pesticide on plant tissues. To use an effective pest control practice and to provide fresh pesticide-free produce at market, it is important to know the degradation rate of a pesticide on plants grown in the field. This study evaluated the influence of shade covers on the degradation of carbaryl on field-grown pakchoi in the lowland humid tropics.

## Materials and methods

**Experiment setup and plant sampling.** The experiment was conducted at a commercial farm near the Guam Agricultural Experiment Station in Yigo village, located at long. 144°55'E and lat. 13°33'N. Pakchoi plants were seeded in trays and transplanted on soil classified as Guam cobbly clay (clayey, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents). Plants were grown in 75-cm-wide (29.5-inch) raised beds, having three rows of plants within each bed. Both the inter-row distance and the distance between plants within a row on a raised bed was 25 cm (9.8 inches). The distance between adjacent beds was 120 cm (47.2 inches). Plants were irrigated with a drip irrigation system.

Treatments included covering plants with 1) black woven polypropylene shade cloth (Takii Co. Ltd., Kyoto, Japan), 2) silver woven polypropylene shade cloth (Japan Wide Cross Co. Ltd., Tokyo, Japan), and 3) white woven polypropylene shade cloth (Teijin Co. Ltd., Osaka, Japan). A clear polyethylene film (Mitsubishi Vinyl Co. Ltd., Tokyo, Japan) was placed to shelter plants before setting up these shade-cloth treatments. This film prevented the rain from washing carbaryl off the plants. The fourth treatment used only a polyethylene film to cover plants. A treatment without any covers was also included as control. All covering materials were placed on top of wire tunnel frame with a height of 75 cm and the width of 150 cm (59.1 inches).

The experiment was arranged in a randomized complete block design with five treatments and three replications. Each plot consisted of average of 30 plants with the range of 28 to 32 plants. Before the experiment, two mature leaves from each plot were tested to confirm the absence of carbaryl on plants by ELISA kit (Strategic Diagnostic Inc., Newton, Pa.).

At 1200 HR on 3 Sept. 1999, the light intensity, air temperature and relative humidity under each shade treatment were recorded (Table 1). Both the light intensity and the relative humidity were measured for each plot and one measurement of air temperature was taken for each treatment. The light intensity was recorded with a quantum meter (Spectrum Technologies, Inc., Plainsfield, Ill.). The percent of shade under each treatment was determined the control without any coverings as the reference of 0% shade. The air temperature and the relative humidity were measured with a hygro-thermometer clock (Cole Parmer, Vernon Hills, Ill.).

Daily rainfall and air temperature data were also recorded at an open field near the experimental site at 1200 HR during 3 to 10 Sept. 1999 (Fig. 1). Rainfall data was taken reading a rain gauge that was set up at 1200 HR on 2 Sept. 1999.

Carbaryl at a.i. 10.6 g·L$^{-1}$ (Sevin 80S; Bayer CropScience, Research Triangle Park, N.C.) was applied on 4-week-old transplants with 8 to 10 mature leaves at about 1700 HR on 3 Sept. 1999. The insecticide was mixed with deionized water and applied at a water equivalent of 935.4 L·ha$^{-1}$ (100 gal/acre). A backpack sprayer with diaphragm pump [413.7 kPa (60 lb/inch$^2$)] and a fine flat fan nozzle was used. A pH of the pesticide solution after mixing was 7.7 and the temperature was 29 °C (84.2 °F). Within an hour after the pesticide application, two fully expanded leaves were sampled from each plot, labeled as 0 DAP (days after pesticide application). Each sample was stored in a cooler, and carbaryl level was measured within 6 h using an ELISA kit (Strategic Diagnostics, Inc.). With the

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**Table 1. Light intensity, percentage of shade, air temperature, and relative humidity recorded for each cover treatment at Yigo, Guam, at 1200 HR on 3 Sept. 1999; °F = 1.8(°C) + 32.**

| Treatment | Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | Shade (%) | Air temp (°C) | Relative humidity (%) |
|---|---|---|---|---|
| Black shade + clear plastic | 461 ± 17.6 | 75 | 28.1 | 77 ± 0.5 |
| Silver shade + clear plastic | 815 ± 74.9 | 56 | 28.5 | 77 ± 0.3 |
| White shade + clear plastic | 1243 ± 180.6 | 33 | 29.1 | 78 ± 0.7 |
| Clear plastic only | 1611 ± 196.2 | 13 | 30.0 | 79 ± 0.3 |
| No covers (control) | 1850 ± 176.9 | 0 | 32.0 | 80 ± 0.5 |

The values for light intensity and relative humidity represent the mean and the standard deviation of three replications. The percent (%) of shade was calculated by the equation, 100 – (light intensity of a shade treatment)/(light intensity of control) × 100.

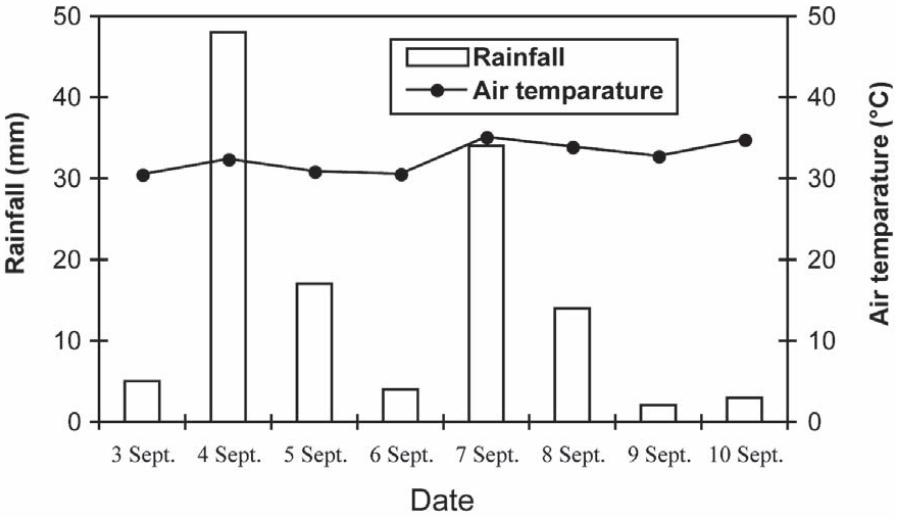



**Fig. 1. Rainfall and air temperature recorded at the experimental field (Yigo, Guam) at 1200 HR from 3 to 10, Sept. 1999; 25.4 mm = 1.0 inch, °F = 1.8(°C) + 32.**

same method, two fully expanded leaves sprayed with carbaryl were sampled from each plot on 1, 3, 5 and 7 DAP to determine the pesticide residue.

**ENZYME-LINKED IMMUNOSORBENT ASSAY FOR CARBARYL.** Ten grams of composite fresh leaf samples was homogenized in 20 mL of acetone at the medium speed for 60 s with a homogenizer (Cole-Parmer). The top 4-mL acetone layer of homogenate was transferred to a tube containing 1 to 2 g of polyvinylpolypyrrolidone (PVPP) (Sigma Chemical Inc. St. Louis, Mo.) and vortexed for 15 s. Two grams of salt reagent (Strategic Diagnostics, Inc.) was then added to each tube and allowed to stand for 5 min for the acetone aqueous phases to separate. The acetone top layer (50 µL) was transferred into a 13 × 100 mm glass tube and was evaporated to dryness using nitrogen gas. The sample was redissolved in 2.5 mL of RaRID assay sample diluent (Strategic Diagnostics, Inc.) and stored at 4 °C (39.2 °F).

Carbaryl antibody coupled paramagnetic particle solution (500 uL) (Strategic Diagnostics, Inc.) was added to each test tube containing 200 uL of sample and 250 uL of carbaryl enzyme conjugate (Strategic Diagnostics, Inc.). After vortexing for 1 to 2 s, the content was incubated for 20 min at room temperature of 25 °C (77.0 °F) and was placed in a magnetic separation rack for 2 min. The solution was decanted and the sample was washed twice with 1 mL of buffer solution (Strategic Diagnostics, Inc.). Color solution (500 µL) (Strategic Diagnostics, Inc.) containing hydrogen peroxide and 3, 3', 5, 5'-tetramethylbenzidine was added to the sample, vortexed for 2 s, and incubated at room temperature for 20 min. The amount of carbaryl in each sample was recorded within 15 min after adding 500 µL of stopping reagent (Strategic Diagnostics, Inc.) using a spectrophotometer at 450 nm (U-2000; Hitachi High Technologies, San Jose, Calif.).

**DATA ANALYSIS.** The half-life of carbaryl was calculated as described by Aly and El-Dib (1971) and Denton et al. (1995). After log transformation, the concentration of the pesticide (dependent variable) was plotted against the date of sampling (independent variable) for each shade treatment. The half-life of carbaryl was then calculated using the equation, $t_{1/2} = 0.693/K$, where $t_{1/2}$ = the time taken for 50% of the pesticide to degrade and K = the rate loss constant. The value K was obtained after multiplying the slope of the regression equation by 2.303 (Aly and El-Dib, 1971).

The slopes of log transformed regression lines for all treatments were compared by an analysis of covariance (ANCOVA) (Snedecor and Cochran, 1967; Sokal and Rohlf, 1969). The analysis of covariance revealed that both the level of shade (a factor) and the number of days after carbaryl application (a covariate) might affect the amount of pesticide residue on plant tissues (dependent variable). A posthoc test (Student-Newman-Keuls test) was applied to determine the degree of similarity of the slopes of regression lines affected by the shade treatment at 0.05 probability level.

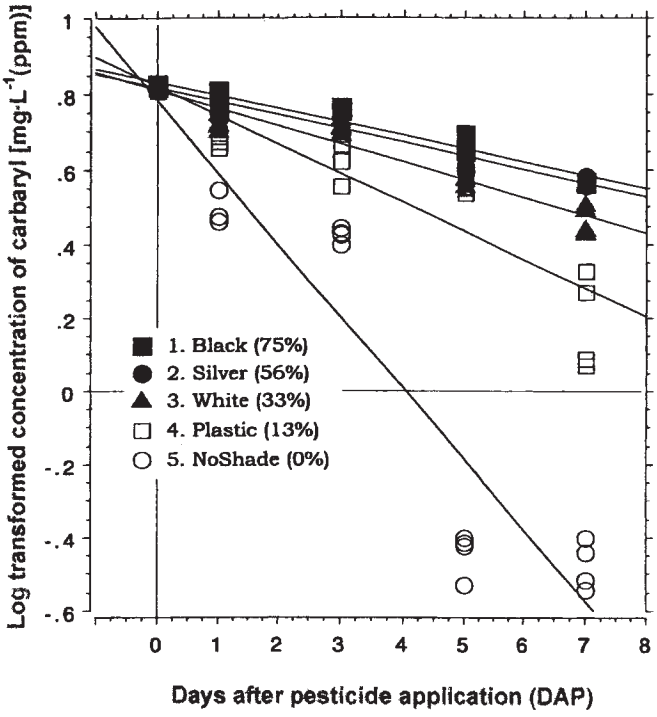
The shade treatments were also compared on each sampling date by subjecting the original data to analysis of variance (ANOVA). Treatment means were also compared by a post hoc test (Fisher's protected least significant difference) at 0.05 probability level. On each examined dates of 1, 3, 5, and 7 DAP, both linear and quadratic regression equation of carbaryl residue (dependent variable) against the percentage of shade cover (independent variable) were determined to study the relationship of two variables. All analyses were conducted using StatView version 5.01 (SAS Institute, 1998).

## Results and discussion

The half-life of carbaryl on field-grown pakchoi ranged from 2 to 9 d depending on the shade treatment (Table 2). In the open field with 0% shade (control), the half-life of carbaryl was the shortest with 2 d. The half-life of carbaryl was 9 d under shade treatments

**Table 2. The half-life of carbaryl on pakchoi leaves grown at Yigo, Guam, under each cover treatment. Carbaryl at a.i. 10.6 g·L$^{-1}$ (1.42 oz/gal) was applied on 4-week-old transplants on 3 Sept. 1999.**

| Treatment | Shade (%) | Half-life of carbaryl (d) |
|---|---|---|
| Black shade + clear plastic | 75 | 9 |
| Silver shade + clear plastic | 56 | 8 |
| White shade + clear plastic | 33 | 6.5 |
| Clear plastic only | 13 | 4 |
| No covers (control) | 0 | 2 |




| Treatment | Shade (%) | Regression equation[z] | $r^2$ |
|---|---|---|---|
| 1. Black shade + clear plastic | 75 | Y=(0.035)X + 0.832 | 0.935 |
| 2. Silver shade + clear plastic | 56 | Y=(0.036)X + 0.815 | 0.921 |
| 3. White shade + clear plastic | 33 | Y=(0.047)X + 0.810 | 0.931 |
| 4. Clear plastic only | 13 | Y=(0.077)X + 0.821 | 0.818 |
| 5. No shade | 0 | Y=(0.194)X + 0.784 | 0.896 |

[z]Y= log transformed concentration of carbaryl recovered in mg·L$^{-1}$, and X = DAP.

**Fig. 2. Log-transformed concentration of carbaryl detected on pakchoi leaves on 0, 1, 3, 5 and 7 d after pesticide application (DAP). Each linear regression line presents the relationship of pesticide residue and DAP for each treatment. The active ingredient of carbaryl at a.i. 10.6 g·L$^{-1}$ (1.42 oz/gal) was applied on 4-week-old transplants on 3 Sept. 1999.**

of the black cloth + plastic cover (75% shade), 8 d for the silver cloth + plastic cover (56% shade), 6.5 d for the white cloth + plastic cover (33% shade), and 4 d for the treatment of plastic cover only (13% shade).

Since the linear regressions of log-transformed carbaryl amount (dependent variable) against DAP (independent variable) were significant for all shade treatments (Fig. 2), the analysis of covariance was conducted to measure a similarity of those regression equations as an influence of the shade treatment. The analysis revealed that the treatment affected the slopes of regression lines, suggesting that shading influenced the rate of carbaryl breakdown on plant tissues. The results from a post hoc test revealed that the slopes of regression lines for the 75% and 56% shade treatments were not significantly different ($P$ = 0.787) suggesting that the breakdown rate of the insecticide was very similar under the two highest shade covers. Likewise the slopes of regression lines for the 56% and 33% shade treatments were not significantly different ($P$ = 0.115). All other pair comparisons of shade treatments showed that the slopes of the regression lines were significantly different ($P < 0.05$) suggesting that% shade was a factor influenced the rate of chemical loss from plant tissues. Control without any covers had the most rapid loss of carbaryl, followed by the 13% shade cover where plants were grown under clear plastic cover only.

The ANOVA was also conducted to determine an effect of shade treatments on pesticide residue for each sampling date (Table 3). On all sampling dates, the mean carbaryl concentration was highest for the 75% shade treatment. This value was not significantly different from the carbaryl concentrations detected from the 56% treatment of (Table 3). On 1 DAP plants grown under the 75% and 56% shades retained more than 90% of the original amount of carbaryl, recording 6.08 mg·L$^{-1}$ (ppm) and 5.70 mg·L$^{-1}$, respectively. On the other hand, the least amount of the pesticide (3.10 mg·L$^{-1}$) was detected from leaves of plants grown under the control. Chemical loss in the control seemed to be due to both light and rain wash off. Nearly 48.3 mm (2 inches) of rainfall recorded during 24 h between the pesticide application and the first sampling time (Fig. 2) possibly accelerated the pesticide loss for control on 1 DAP. The breakdown of the insecticide on plants without covers was very rapid having only 0.36 mg·L$^{-1}$ of carbaryl detected in plant tissues on 5 DAP.

Under other four cover treatments ranging from 13% to 75% shade, the pesticide loss due to the rainfall was prevented by the protective plastic covering. Data in Table 3 exhibits that as the percentage of shade increased, the higher concentration of carbaryl remained on plants on all sampling dates. When regression analyses were applied, it was found that on each examined dates of 1, 3, 5, and 7 DAP, both linear and quadratic regression equation of carbaryl residue (dependent variable) against the percentage of shade cover (independent variable) were highly significant ($P < 0.001$).

The study clearly showed that the

**Table 3. Mean carbaryl concentration (n = 3) recovered from pakchoi grown at Yigo, Guam, under each cover treatment on 1, 3, 5, and 7 d after pesticide application (DAP). Carbaryl at a.i. 10.6 g·L$^{-1}$ (1.42 oz/gal) was applied on 4-week-old transplants on 3 Sept. 1999.**

| Treatment | Shade (%) | Concn of carbaryl recovered [mg·L$^{-1}$ (ppm)] 1 DAP | 3 DAP | 5 DAP | 7 DAP |
|---|---|---|---|---|---|
| Black shade + clear plastic | 75 | 6.08 a | 5.78 a | 4.70 a | 3.68 a |
| Silver shade + clear plastic | 56 | 5.70 a | 5.61 a | 4.02 ab | 3.73 a |
| White shade + clear plastic | 33 | 5.25 b | 5.15 a | 3.72 b | 2.95 a |
| Clear plastic only | 13 | 4.80 c | 4.34 ab | 3.56 b | 1.60 b |
| No covers (control) | 0 | 3.10 d | 2.66 b | 0.36 c | 0.34 b |
| Average | | 4.99 | 4.71 | 3.27 | 2.46 |

Means followed by the same letter within each DAP are not significantly different by the Fisher's protected least significant difference at $P = 0.05$.

amount of carbaryl residue detected on pakchoi leaves was greatest under the darkest shade cover. The combined environmental factors of sunlight (Table 1) and precipitation (Fig. 1) might have intensified the reduction of the pesticide on plants grown without any coverings. The reduced light penetration plus no rain effects led to longer half-life of the insecticide under the shade covers.

This result complements reports of faster degradation of two insecticides, malathion and diazinon, on okra (*Abelmoschus esculentus*) and sweet pepper (*Capsicum annuum*) when grown in open field on Guam compared to those plants grown in shaded area (Demeterio, 1981 and 1983). The result of the present study also agrees with the report by Crosby (1969) indicating that light intensity greatly influenced pesticide degradation. It is probable that since the shade covers lowered the penetration of light, the lower light intensity was responsible for a reduction in the rate of volatilization and hydrolysis of the pesticide, thus resulting in prolonging the pesticide presence.

This study concluded that carbaryl degraded faster in an open-field compared to low light intensity treatments in the tropics. Understanding that the fate of a pesticide depends greatly on a plant's growing environment enables more effective pest management practices.

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