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DETERMINATION OF LAMBADA AND THIAMETHOXAM RESIDUES IN SQUASH FRUITS AND LEAVES

Shaimaa G. Lasheen[1*], R.M. Sherif[1], M.A.I. Youssif[1] and H.M. Sallem[2]

[1*]Plant Protection. Departmentt., Faculty of Agriculture., Zagazig University., Egypt.
[2]Plant Protection. Institute., Argiculture. Research. Center., Dokky, Giza, Egypt.

Abstract

Lambda cyhalothrin and Thiamethoxam were used by the recommended rate, which were (1 lit / fedd.) on squash plants. The residues were determined after 1, 3 and 7 post treatments.

The residual effects were determined on leaves, washed fruits and unwashed fruits. However the amount of these residues were as follows: 5.7, 3.2 and 6.5; 1.8, 0.8 and 3.1; 0.29, 0.04 and 0.37 for Lambda cyhalothrin at the three mentioned periods for the two tested seasons respectively. while, the residual effects were 4.6, 2.4 and 4.9; 1.2, 0.55 and 1.8 ; 0.18, 0.026 and 0.17 for Thiamethoxam at the same periods, respectively.

There was a positive correlation between the figures of Lambda cyhalothrin residues in squash fruits and the efficiency of washing process. The role of washing with tap water in physical removing of Lambda cyhalothrin form squash fruits surfaces ranged between 5 to25 percent.

Amount of thiamethoxam residues in unwashed fruits were 4.6, 1.2 and 0.18 after one hour, 3 and 7 days after spraying, respectively. Dissipation percentages were 15 and 26.1 % after the corresponding preceding periods from spraying.

As well as results showed the residues of thiamethoxam in washed fruits were 2.4, 0.55 and 0.026 after one hour, 3 and 7 days after spraying, respectively. The loss percentages ranged between 22.9 to 4.7%.

Key words: Lambada, Thiamethoxam, Residues, Squash Fruits, Leaves.

Introduction

Pesticide residue refers to the pesticides that may remain on or in food after they are applied to food crops, defined by WHO, (2016) as any substance or mixture of substance in food for man or animals resulting from the use of pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products and impurities that are considered to be of toxicological significance.

Most pesticides are resistant to physical, chemical and biological degradation and accumulate in both aquatic and terrestrial food webs (Valiyaveettil *et al.,* 2010). Over 98% of sprayed insecticides and 95% herbicides reach a destination other than their target species, including non-target species, air, water and soil. Pesticide residues are very small amounts of pesticides that can remain in or on a crop after harvesting or storage and make their way into food chain (Food Standards Agency, 2010).

They can remain even when pesticides are applied in the right amount and at the right time. Pesticide residue refers not only to the active ingredient but also to any derivatives of pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance (Hamilton and Crossley, 2004).

More than700 pesticides are registered for use in the world and more continue to persist in the environment even though they are no longer being applied (Wylie, 1997). Persistent pesticides are highly toxic, causing an array of diverse effects, notably death, diseases and birth defects among humans and animals (Etonihu *et al.,* 2011).

Pesticides are used on fruits, vegetables, wheat, rice, olives, canola pressed into oil and on non-food crops such as cotton, grass and flowers. However pesticides applied to food crops in the field can leave potentially harmful residues (Botwe *et al*., 2011). According to Keikotlhaile and Spanoghe, (2011), after pesticides are applied to the crops, they may interact with the plant surfaces, be exposed to the pesticide residues causing damage.

Pests and plant diseases have been a problem to vegetable farmers and a threat to food security globally as they destroy crops and reduce yields hence financial losses. Pests are organisms that feed on plants as a source of food (Ata *et al*., 2013). Pests can be carriers of plant pathogens which also exist in soil. Farmers use pesticides to control the pests and plant diseases. Pesticides have been in use since early years by Sumerians where they used compounds of sulphur to control mites and insects 4500 years ago. They also used mercury, lead, zinc and arsenical compounds to grow vegetables and fruits (Unsworth, 2010).

Material and Methods

Spraying of Lambda cyhalothrin and thiamethoxam in the field

This experiment was carried out at new canal village, AL-Ebrahemia district, Sharkia government during 2018 squash growing seasons. The mature plants were sprayed once with Lambda cyhalothrin and thiamethoxam using at the recommended field rate (1L/ fed and 20g / l00 Lwater), respectively.

Sampling

Squash leaves and fruits samples were collected at random from treated and untreated plants after one hour of application and then after 3 and 7 days post- treatment. Samples were taken from bottom, mid and top of the fruits and leaves after that the sample immediately transported to laboratory in plastic bags after collection. Each fruits samples was divided into two subsamples the first one was left unwashed and the second one was washed with tap water and left for air dryness. Each fruits sub-sample (100 g) as well as the sub-sample of leaves (25 g) was subjected to extraction and clean up procedures.

Extraction and clean-up

• Extraction:

Lambda cyhalothrin and thiamethoxam residues were extracted from the plant samples according to the method of (Machell *et al*., 1975). A quantity of 100 gm and 25 of chopped samples and 25 gm of anhydrous sodium sulphate were blended with 200 ml of methyleen chloride for 5 min. the mixture was filtered through filter paper containing anhydrous sodium sulphate. 100 ml of the extracted solution, which represents 50 gm of the intial sample, was collected. The solvent was evaporated to near dryness using air.

The residues were then transferred quantitatively into a small glass vial by 10 ml of acetone. The solvent was evaporated to dryness on water bath at 35-70c and the residues were dissolved in 0.5 ml acetone.

• Clean-up:

Glass plates (20 × 20 cm) coated with silica gel GF; silica gel was dispersed in distlled water at 1:2 w/v. fribos applicator was used for coating the glass plates with a thin layer (0.25 mm thickness). The plates were then put in the oven adjusted at 110c for one hour. An aliquot (0.1 ml) of the concentrated extract was spotted on the plate at a distance of 3 cm from the lower edge. The standard active ingredient from each insecticide sample was also spotted on the same plate in order to define the RF values. The plates were developed in hexane acetone (7:3 v/v), then exposed to u.v. light in order to detect the spots of the authentic sample to calculate thr RF values of the tested insecticide. The spots were scraped from the plate and the insecticides residues were extracted by acetone using a centrifuge. The solvent was than decanted and evaporated to dryness. The residues were determined colorimetrically.

Determination of Lambda cyhalothrin and thiamethoxam residues

High Performance Liquid Chromatographic (HPLC) Analysis. Extracted samples of vegetables were analyzed by high performance liquid chromatography (HPLC) following the method of (Ohlin, 1986) and (Hiemstra *et al*. 1999). HPLC analyses were performed in isocratic system using a Perkin Elmer Chromatograph including Series 200 pump, Series 200 UV/VIS detector and a Supelco C18 analytical column (25 cm × 4.6 mm (i.d)). Acetonitril /water was used as mobile phase. 20 µl sample was injected through auto sampler. The column temperature was kept 30ºC with a flow rate of 1ml min-1.

Recoveries of Lambda cyhalothrin and thiamethoxam from different samples through the extraction and clean-up procedures

The recoveries of Lambda cyhalothrin and thiamethoxam from different plant samples through the extraction and clean-up procedures were estimated. Untreated plant materials were fortified with the tested insecticide. The fortified samples were subjected to the extraction and clean-up steps and recoveries of insecticide were detremind chemically. Results of Lambda cyhalothrin and thiamethoxam residues were corrected using its respective recovery in squash fruits and leaves which were 88 and 85%, respectively.

Results and Discussion

Residues of the insecticide Lambda cyhalothrin on / in squash fruits and leaves

The mature squash was sprayed with the recommend

Table 1: Residues of Lambda cyhalothrin in squash fruits and leaves after different periods.

Days after spraying	Un washed fruits		Washed fruits		Leaves	
	Ppm	% loss	Ppm	% loss	Ppm	% loss
1h.(initial)	5.7	-	3.2	-	6.5	-
3 days	1.8	31.6	0.8	25	3.1	51.6
7 days	0.29	16.1	0.04	5	0.37	11.9

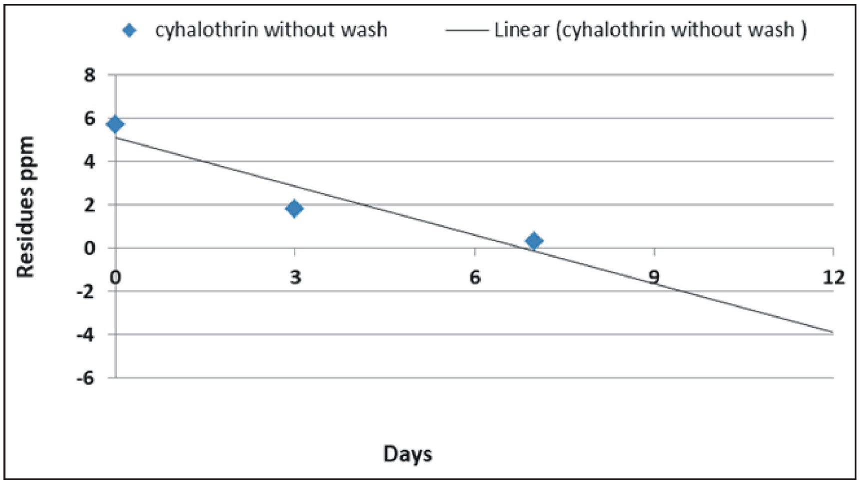

Fig. 1: Cyhalothrin unwashed in squash.

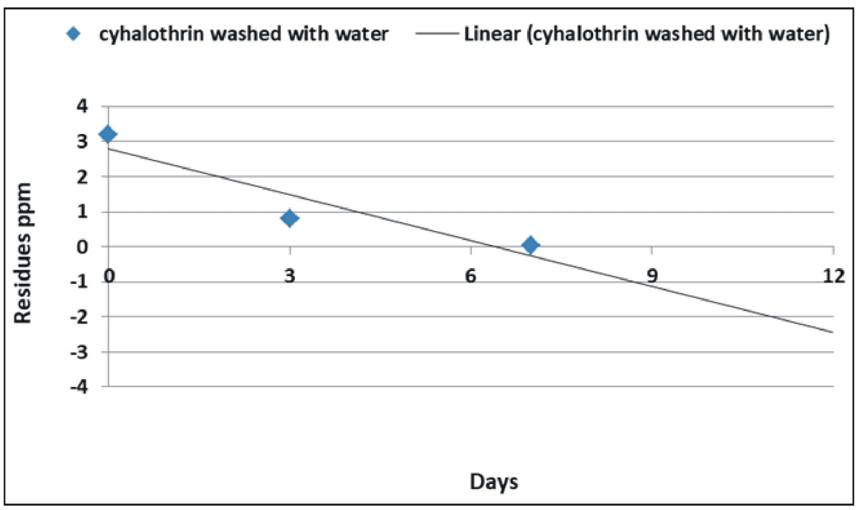

Fig. 2: Cyhalothrin washed with tap water in squash.

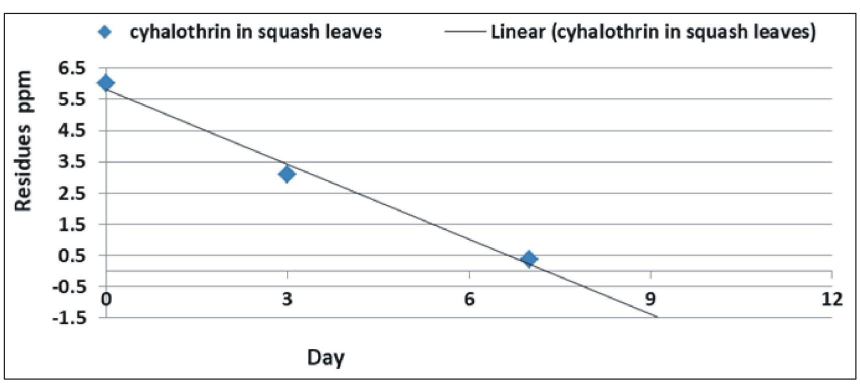

Fig. 3: Cyhalothrin in squash leaves.

rate. For residue analysis, samples of the sprayed plants (fruits and leaves) were collected at random from treated plants as well as unsprayed ones immediately one hour after spray and then after 3 and 7.

Results presented in table 1 and fig. 1 show the residue dissipation in Lambda cyhalothrin contaminated squash fruits and leaves. Summarized results showed that initial amount of Lambda cyhalothrin in unwashed fruits, as deteramined after one hour of spraying, was 5.7 mg a.i. / kg squash fruits. However the amount are decreased to 1.8 and 0.29 after 3and 7 days post- treatment, respectively. The corresponding values of the loss percentages based on the initial amounts, were 31.6 and 16.1%.

Also, the results showed that amount of Lambda cyhalothrin in washed fruits after one hour, 3 and 7 days post -treatment was 3.2, 0.8 and 0.04, respectively. While the loss percentages of Lambda cyhalothrin residues, due to washing squash fruits ranged between 25 to 5%.

There was a positive correlation between the figures of lambada cyhalothrin residues in squash fruits and the efficiency of washing process. The role of washing with tap water in physical removing of lambada cyhalothrin form squash fruits surfaces in presented in table 1.

On the other hand the amount of lambada cyhalothrin residues in sprayed squash fruit in unwashed were much higher than those detected in sprayed fruits after one hour, 3 and 7 days were 5.7, 1.8 and 0.29 mg a.i./kg. The corresponding loss percentages were 31.6 and 16.1%. Also, the amount of lambada cyhalothrin residues in sprayed squash fruit in washed were much higher than those detected in sprayed fruits after one hour, 3 and 7 days were 3.2, 0.8 and 0.04 mg a.i./kg. The corresponding loss percentages were 25 and 5%.

Table 2: Residues of thiamethoxam in squash fruits and leaves after different periods.

Days after spraying	Un washed fruits		Washed fruits		Leaves	
	Ppm	% loss	Ppm	% loss	Ppm	% loss
1h.(initial)	4.6	-	2.4	-	4.9	-
3 days	1.2	26.1	0.55	22.9	1.8	36.7
7 days	0.18	15	0.026	4.7	0.17	9.4

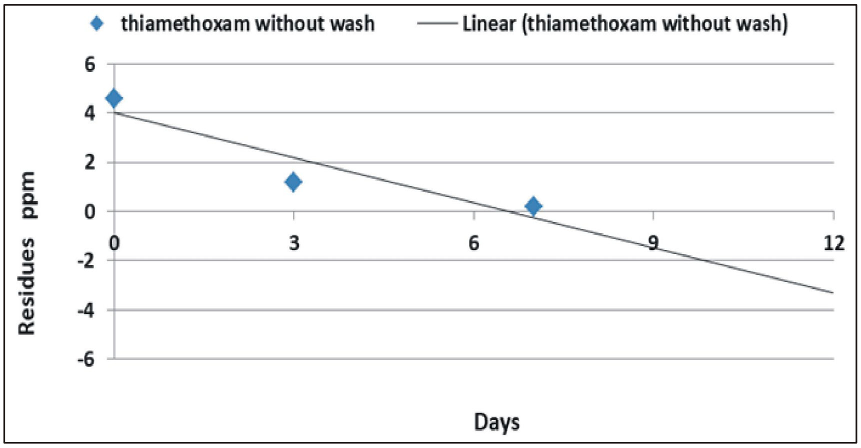

Fig. 4: Thiamethoxam residues in unwashed in squash.

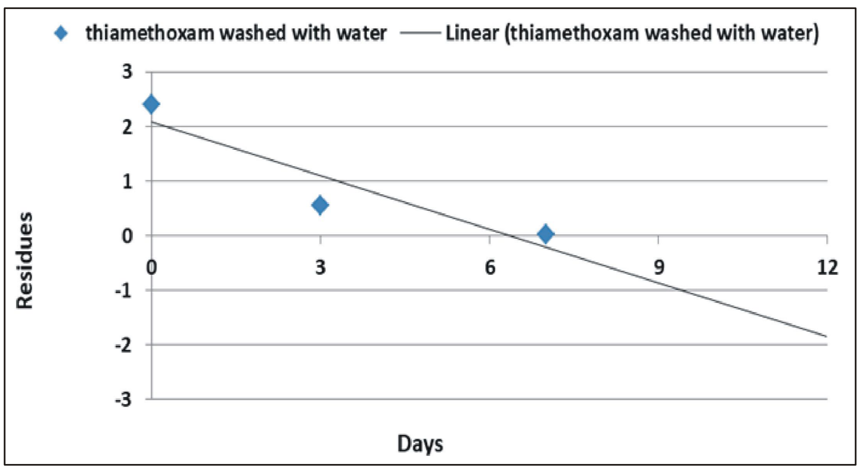

Fig. 5: Thiamethoxam residues in washed squash with tap water.

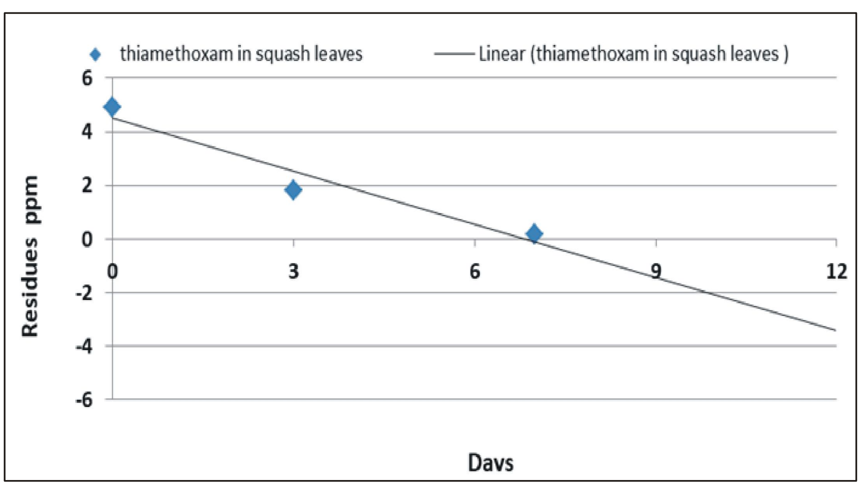

Fig. 6: Thiamethoxam residues in squash leaves.

Regarding the amount of lambada cyhalothrin residues in sprayed leaves were much higher than those detected in sprayed fruits. Amounts of lambada cyhalothrin after one hour, 3 and 7 days were 6.5, 3.1 and 0.37 mg a.i./kg leaves. The corresponding loss percentages were 51.6 and 11.9%.

It is worth to mention that the maximum residue limit of lambada cyhalothrin on squash fruits is (0.5 mg /kg b.w. daily) (CAC, 2003). Data presented in table 1 show that lambada cyhalothrin sprayed squash fruits could be, however, harvested and marketed for human consumption directly after spraying.

Residues of the insecticide thiamethoxam on / in squash fruits and leaves

Results showed the residue of thiamethoxam in table 2 in /on squash fruits and leaves. Amount of thiamethoxam residues in unwashed fruits were 4.6, 1.2 and 0.18 after one hour, 3 and 7 days after spraying, respectively. Dissipation percentages were 26.1 and 15% after the corresponding preceding periods from spraying.

As well as results showed the residues of thiamethoxam in washed fruits were 2.4, 0.55 and 0.026 after one hour, 3 and 7 days after spraying, respectively. The loss percentages ranged between 22.9 to 4.7%.

Regarding the amount of thiamethoxam residues in sprayed leaves were much higher than those detected in sprayed fruits. Amounts of thiamethoxam after one hour, 3 and 7 days were 4.9, 1.8 and 0.17 mg a.i./kg leaves. The corresponding loss percentages were 36.7 and 9.4%.

Data of thiamethoxam sprayed squash leaves showed that amount of thiamethoxam residues were 4.9, 1.8 and 0.17 mg /kg leaves after one hour, 3 and 7 days, respectively loss percentages were 36.7 and 9.4%.

Table 3: Impact of lambada cyhalothrin and thiamethoxam residues squash fruits (washed and unwashed) and leaves.

Treatments	Washed & unwash squash fruits and leaves		
	Slope	K	T 1/2 (days)
lambada cyhalothrin without wash in squash fruit	0.28	0.64	1.08
lambada cyhalothrin washed with water in squash fruit	0.27	0.62	1.12
lambada cyhalothrin in squash leaves	0.41	0.94	0.74
thiamethoxam without wash in squash fruit.	0.22	0.51	1.36
thiamethoxam washed with water in squash fruit.	0.27	0.62	1.12
thiamethoxam in squash leaves	0.26	0.59	1.17

During the corresponding preceding intervals.

It is worth to mention that the maximum residues level of thiamethoxam on squash fruits is (1.4 mg/kg b.w. daily) in (CAC, 2003).

In order to study the rate of degradation and half-life period of the insecticide lambada cyhalothrin and thiamethoxam , the following steps were made:

• The relative between the logarithm of ppm record and time intervals were plotted.

• A straight line was fitted by eye, then the slop of this line was calculated.

• The rate of constant (i.e. rate of degradation) was optained from the following equation: Rate of degradation (k) = 2.303*slope.

• Finally, the half- life period (t 1/2) was obtained from the following equation : t 1/2 = 0.693 /k.

The calculated values of the rate of decomposition (k) and the half-lifes periods of lambada cyhalothrin and thiamethoxam in squash (unwashed, washed fruits and leaves) are presented in table 3.

Data showed that dissaperace of lambada cyhalothrin and thiamethoxam due to degradation in fruits of 2 tested plants was higher compared with leaves. This phenomenon was more noticeable with 7 days. The half-lifes values were 1.08, 1.12 and 1.36, 1.12 days in squash (unwashed and washed) fruits, respectively. The corresponding figures of lesves were 0.74 and 1.17 days.

The response of each pesticide was different in relation to their thermal stability, fragmentation and volatilization, so was necessary to get to a compromise based on the obtained results. In the case of carbaryl, it was affected with all the temperature studied, but it had a positive response on its integration areas until 200ºC; probably, above this temperature carbaryl becomes thermally unstable. It is known, that carbamates are thermally sensible and there are prone to degradation (Przybylski and Bonnet, 2009). Possibly, that is the reason associated with the appearance of 1-naphthol in our chromatogram; this compound could be produced due to the existence of fragile bonds on the carbaryl structure and/or by hydrolysis (Przybylski and Bonnet, 2009).

Farmers used different types of pesticides where among the fungicides dithi-ocarbamites and, pyrethroids were the most used among the fungicides and insecticides, respectively. Mdegela *et al.*, (2013) reported as well a high proportion and quantity of pyrethroid for pesticides used at Mindu dam catchment area in Morogoro. All the registered pesticides used by farmers in Tanzania are in class II which are moderately hazardous (TPRI, 2007; WHO, 2009). When carefully handled Class II pesticides are considered to be of low health effects.

The essence of pesticide monitoring is to ensure that pesticides in fruits and vegetables do not exceed maximum residue levels (MRLs) allowed by the government, no misuse of pesticides that could result in unexpected residues in food and that good agricultural practices (GAP) are maintained (Keikotlhaile and Spanoghe, 2011). Pesticide residue analysis is tremendously an important process in determining the safety of using certain pesticides (Dasika *et al.*, 2012). Pesticide residues in fresh produce are a major consumer concern (Keikotlhaile *et al.*, 2010).

The exposure could be in the field or at storage sites. The variation in the levels of the pesticide residues detected in the tissues of the vegetables from the different markets could be markets could be attributed to difference in the level and type of pesticide use at the various locations that the vegetable originated from. This is in consonance with earlier studies by Botwe *et al.*, (2011); Petersen *et al.*, (2013) that reported differences in pesticide residues in foods items from different locations. Some health implications have been associated with pesticide residues in food ranging from headaches and nausea to chronic impactslike cancer, reproductive harm and endocrine disruption and others (Recio-vega *et al.*, 2008; Berrada *et al.*, 2010; Gilden *et al.*, 2010; Chiu *et al.*, 2015), however the risk analysis result obtained in this study seem to suggests that there is no risk as at the time of the study on the consumption of those vegetables.

The maximum residue levels for Cypermethrin as pyrethroides in collards, swiss chard and tomatoes are

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1mg/kg, 2mg/kg and 0.2mg/kg respectively (FAO/WHO, 2009). Collard greens from Soko-mjinga (1.397±0.478 mg/kg), swiss chard from Soko-mjinga (2.458±0.298mg/kg) and Ponda-mali (2.495±0.609mg/kg) and tomatoes from all the three markets exceeded the recommended level. Swiss chard from the two markets were 1.229 and 1.248 times higher than the recommended level respectively. Tomatoes from Soko-mjinga, Ponda-mali and Main Municipal Market markets had residues levels that were 1.16, 1.48 and 2.005 times higher the recommended level respectively.

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