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# Investigating the Effects of Formulation, and Geographical Location on Degradation of Carbendazim in French Beans, Kenya

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***Abstract:***
*This study was conducted in Naivasha and Kabaa regions of Kenya during short and long rain seasons to determine the effects of difference in formulation and geographical location on the degradation and pre-harvest interval of carbendazim pesticide in French beans. Pre-harvest interval (PHI) ensures food safety after application of pesticide. Serengeti French beans variety; Chariot 500 SC and Rodazim SC carbendazim formulation were used. The pesticide was applied at the rate of 625g of carbendazim per hectare before harvest and the French beans samples were collected on 0, 3, 7, 14 and 16 days after pesticides applications, analysed using liquid chromatography technique with a triple quadruple mass detector (LCMSMS). Carbendazim degradation in the first three days after the pesticide application was sharp regardless of the formulation used, season or geographical location. The percentage degradation of carbendazim showed insignificant variation regardless of the initial concentration. There was more than 96.7% reduction of carbendazim residues on the 14th day after pesticides applications. Two-way ANNOVA was used to compare the breakdown of carbendazim based on its formulation and geographical locations. The F-critical values (F) obtained were $0.02 < 4.14$ and $0.17 < 2.9$ for formulation and geographical locations respectively at 95% confidence level. The results show that there is no statistical significance difference in breakdown of carbendazim in French beans within the formulations and geographical locations. The half-life of carbendazim was calculated using Langmuir-Hinshelwood kinetic model equation. The results obtained indicate that carbendazim had a dissipation half-life of 1.7 days and 5 day pre-harvest interval calculated at 50% of the 200µg/kg.*

***Keyword:*** *French beans, carbendazim, formulations, Kabaa, Naivasha, Kenya*

## 1. Introduction

The geographical position of Kenya in the tropical zone gives her favorable climatic conditions for the growth of French beans throughout the year with quality attributes such as softness and no stringy fibrous texture. French bean is a vegetable crop under the horticultural sub-sector of agricultural sector. The sub-sector has grown significantly since 2000 to become a major foreign exchange earner to the country (Ebony, 2001; EPZ, 2005). Its contribution to the economy of Kenya has seen it become the third foreign exchange earner after tea and coffee (Odero,2012); providing employment, increased food security and sustainable development in the country. In 2013, the value of horticultural exports from Kenya was Ksh. 93.3 billions (Euros 850 million); more than 70% going into Europe (Odero *et. al*., 2012; HCDA, 2013). French bean is a major vegetable in the sub-sector (Nderitu *et. al.,* 2007) grown mainly for export, with EU being the biggest market as well as for other horticulture product.

In Kenya, small scale farmers (Monda *et al.,* 2003) in the rural areas are the primary producers of fruits and vegetables for both local and export market. French bean is relatively a high value vegetable with high return per hectare, short growth cycle and minimum capital investment, therefore becoming a preferable commercial crop of choice for many small scale farmers, estimated at 50,000

famers (Netherlands Development Organization (SNV), 2012) The change in tropical and global climate due to human activities has significantly increased pests and diseases burden on agriculture crops (Juliian, 2015). This has increased the use of pesticides to control pest and diseases to improve the yield and quality of the produce. On the other hand, meeting the European Union food safety standards in the use of pesticides poses a challenge to the small scale farmers in Kenya.

Carbendazim (methyl benzimidazol-2-yl carbamate (IUPAC) is a systemic benzimidazole fungicide with protective and curative action (APVMA, 2009). It is a common fungicide in the world (Agnieszka *et al*, 2008) used for the control of diseases caused by a broad range of fungi such as Angular leaf spot, powdery mildew, scorch, Fusarium root rot and blight, affecting vegetables, fruits and field crops. It is registered in Kenya as an active ingredient in the commercial formulation; Bendazim 500 SC, Chariot 500 SC, Megaprode Lock 52.5 WP, Pearl 500 SC, Rimeta Gold 300 SC, Rodazim SC, Saaf WP, Exempocurve 250 SC and Sherrif 75 WP(PCPB, 2014). Pre-harvest interval (PHI) is one of the most important aspects for good agricultural practice (GAP) which is key in ensuring the compliance of food commodity with maximum residue levels (MRLs). PHI is the number of days that must pass between the last application of plant protection product and the time of re-entry or harvest of the commodity. The interval is based on the number of days it takes for the applied pesticide to break down in or on the plant to a level when it has no significant impact on the health of the consumer. Therefore, the interval varies greatly, depending on the crop kind and the active molecule applied. A list of registered pesticides under Pest Control Products Board (PCPB) show that carbendazim formulations from different manufacturers for foliar use in control of fungal disease in French beans have varying PHI values. This variation may causes challenges to small scale farmer.

The purpose of this study was to investigate the break down pattern of carbendazin in Chariot 500 SC and Rodazim formulations used in French beans and find the PHI for all registered carbendazim formulation in Kenya.

## 2. Materials and Methods

### *2.1. Studyarea and Site Selection*

Two sites were selected at Naivasha in Nakuru and Kabaa in Machakos counties (Figure 1).These sites represent major French beans growing areas in Kenya for export. The French beans were planted in September, 2015 and March, 2016 to represent short and long rainy seasons respectively. Naivasha site lies between, latitude of 000 45’ S, Longitude 0360 17.7’ E at an altitude of 1,920 m above sea level while Kabaa site lies between latitude of 0010 14.4’ S, longitude of 0370 28.5’ E at altitude of 1,268 m above sea level.

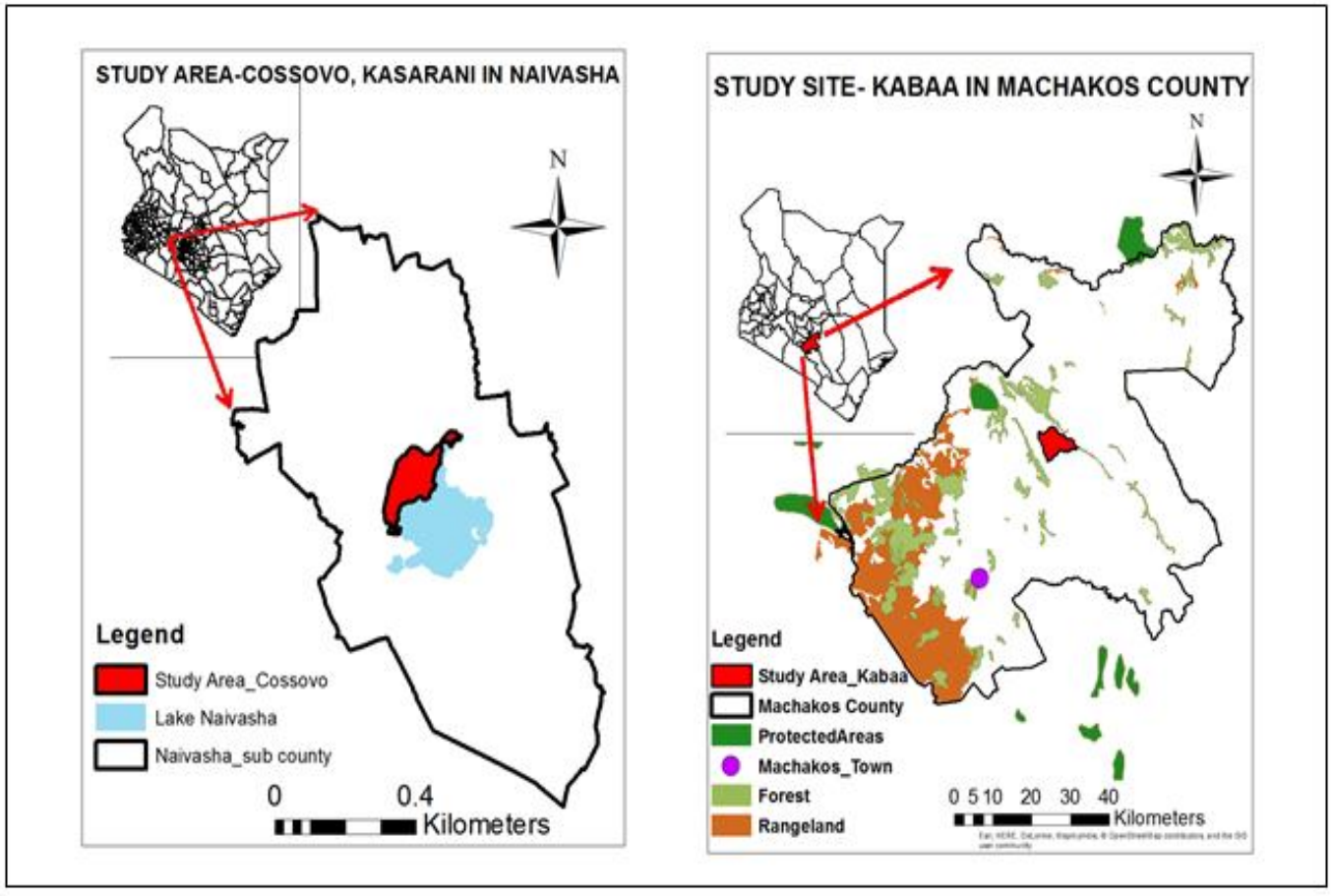


*Figure 1: Map showing location of (a) Naiavasha and (b) Kabaa study sites*

*2.2. Layout of the Field Plots and Site Preparation*

The samples (french beans) were planted in each plot with a total area of $130m^2$(13 m by 10 m) for every treatment separately, giving a total area of $260m^2$. The plots were divided into 4 equal blocks of 2 m x 10m ($20m^2$), three replicate treatments and one control (FAO, 1986) were taken. Adequate buffer zone of 1 m was left in between each of the block and around the plot to prevent contamination due to spray drift. The plot and blocks were also uniquely identified with permanent labels containing field identification number, planting date, expected harvesting date and the variety planted. The plots were ploughed and harrowed by atractor. Two ridges of 90 cm wide and about 30 cm high were formed manually in every block. The crops were planted in two double rows with 50cm space between the double rows and 20cm space within the double rows (Kenneth, 2012).

*2.3. French Bean Variety and Pesticides Selection and Application*

Questionnaires were circulated to 20 exporters for the identification of French beans' variety and carbendazim formulation comonly used in the study areas. As per the information obtained, Serengeti French beans and two carbendazim (*Chariot 500 SC and Rodazim SC*) formulations were selected for the study.

500 SC and Rodazim SC formulations containing carbendazim as an active ingredient were purchased from registered agro-chemical dealers in the study areas and applied in a manner that represents the common application technique used by commercial French beans growers, while following the directions specified by the manufactures (Table 1).The two different knapsack spraying equipment were evaluated and calibrated at the two sites before use to ensure right dosage and uniform application of the pesticide that would result in adequate canopy penetration and coverage.

| Trade name | PCPB reg. No. | Active ingredient | g/l | Treatment | Dosage | Recommended rate |
|---|---|---|---|---|---|---|
| CHARIOT 500 SC | PCPB (CR) 1084 | Carbendazim | 500 | application 1 | 20ml per 20L | 0.5 -1.0L per Ha |
| Rodazim SC | PCPB (CR) 0378 | Carbendazim | 500 | application 2 | 20ml per 20L | 0.75L per Ha |

*Table 1: Rate of carbendazim formulations and pesticide application*

The treatment was applied to the test crop at the rate of 20ml per 20 litres of water following the manufacturer's instructions. The application was done once when the pods were ready for harvesting. The amount applied was 10ml in 10litres in 0.008 Ha for the study sites.

*2.4. Sprayed and Controlled Sampling from the Sites*

Duplicate, 1 kg of beans pods that were ready for harvesting were sampled on the 0, 3, 7, 14 and 16 days after the application of the two carbendazim formulations from each sprayed and control sites. At day zero the samples were collected when the crop was dry to avoid contamination of the person taking the sample or cross contamination of samples. Proper sample handling practices were observed i.e. use of clean gloves when harvesting to prevent transfer of pesticide residue from one sample to another or removal of pesticide on the surface. The samples were then temporarily stored in polyurethane cool boxes containing wet ice in the field vehicle for transportation to the Kenya Plant Health Inspectorate Service (KEPHIS) laboratory for extraction and analyses.

*2.5. Sample Processing, Extraction, clean-up and Analysis*

In the laboratory, the frozen samples were cut into small particle sizes of less than 2mm using Stephan chopper and homogenized by warring blander. Each sample was sub-divided into two analytical portions of 50g. The retained samples were kept frozen at $-18^0$C until the end of the study.

10g of the homogenized analytical portion and 5 control samples were weighed by difference into different 50ml single use extraction polyethylene tubes. One of the control samples was fortified with 50 µl of carbendazim standard solution to achieve a spiking level 0.05µg/g. 50 µl of procedural internal standards Dimethoate D6 (10mg/kg) was added to the sample, control samples, spike sample and solvent blank to achieve 0.05µg/g final concentration.

10ml of acetonitrile was added to the main samples, control samples, spike sample (recovery) and solvent blank, the tubes were tightly closed and shaken vigorously by hand. 6.5 g of premixed extraction salts (4g Magnesium sulphate anhydrous, 1g sodium chloride, 1g trisodium citrate disodium hydrogen citrate sesquihydrate) were added to the mixture and vortexed using Wisemix-VM-10 for 1 minute± 10 seconds to disperse the sample into the solvent. Finally the mixture was centrifuged using Universal 320 R for 5 minutes with centrifuge set at 3700rpm and taken for clean-up.

6ml aliquot of the organic phase of the each extract was transferred into a 15ml single use polyethylene centrifuge tube containing 1.05g of pre-mixed clean-up salts (0.15g PSA and 0.9g anhydrous magnesium sulphate). The tubes were vigorously shaken by hand to avoid caking, vortexed for 1 minute ± 10 seconds and centrifuged for 5minutes with the centrifuge set at 3700 rpm.4ml of the extract was filtered through a 0.45µm membrane filter and transferred into centrifuge tubes. 40 µl of 5% formic acid solution in acetonitrile (v/v) was immediately added to the extracts to adjust the pH to approximately pH 5.

To 2mls of the extracts, 20 µl of formic acid (10 µl /ml of sample) and 60 µl of D-sorbitol (30 µl /ml of sample) was added and vortex to mix .500 µl of the mixture was added to 500 µl of HPLC grade water and vortexed to mix. The final extracts (concentration 1g/ml) were transferred to 2ml vial capped and taken for analysis. The samples were analysed using QuChERS (Quick, Cheap,

Efficient, Robust and Safe) multi-residue method of analysis using Liquid Chromatography technique with a triple quadruple mass detector (LCMSMS- Agilent 6430).To ensure quality control, certified reference standard, control samples, spike (recovery), solvent blank, calibration and internal standard were used to monitor the performance of the procedure and instrument during the analysis. Quality Control samples were prepared together with the analytical samples.

*2.6. Quantification and Sample Analysis*

Calibration standards were prepared at concentrations of 0.01, 0.025, 0.05, 0.075 0.1 and 0.2 µg/ml for validation of method (Table 2); 0.01, 0.05 and 0.2 µg/ml for routine analysis using the extract.

| Concentration level | 1000µg/kg standard solution | Sample extract | Water solvent | Total volume |
|---|---|---|---|---|
| 10µg/kg | 10µm | 500µm | 490µm | 1000µm |
| 25µg/kg | 25µm | 500µm | 475µm | 1000µm |
| 50µg/kg | 50µm | 500µm | 450µm | 1000µm |
| 75µg/kg, | 75µm | 500µm | 425µm | 1000µm |
| 100µg/kg | 100µm | 500µm | 400 µm | 1000µm |
| 200µg/kg | 200µm | 500µm | 0 µm | 1000µm |

*Table 2: Calibration curve standard preparation*

*2.7. Detection Limit for Carbendazim*

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantised as an exact value (EMEA, 2006). The LOD of carbendazim was expressed as the analyte concentration required in producing a signal greater than three times the standard deviation of the noise level as determined by empirical approach consisting of measurements were analytically obtained.

*2.8. Quality Control of the Method*

Quality control of the method was based on recovery from a fortified blank sample with carbendazim standard to obtain a final concentration of 50µg/kg and the ratio of the qualifying ion (ion 205) abundance to that of the transition ion (ion 236.0) of Dimethoate D6 internal standard . The samples were fortified with 50µl of carbendazim 1000µg/kg standard stock solution and 50µl of Dimethoate D6 1000µg/kg stock solution. The recovery results were within the recommended range of 70% to 120% (Hill, 2000) and hence the values were not corrected for recovery. The results indicated that the ratio of the qualifying ion (ion 205) abundance to that of the transition ion (ion 236.0) of Dimethoate D6 internal standard was 8.7 which is with the recommended range of 6.7 to 12.4 as per instrument setting.

*2.9. Test for Outliers*

Grubbs' outlier test statistical in Microsoft excel was applied to determine whether any of the observations with minimum or maximum values in the replicate data sets lies were outliers. The data obtained from the LCMSMS were arranged in-order of the sampling points and test for any outliers using Grubbs test at the 95% confidence level. From the Grubbs' test results, none of the obtained data was found to be an outlier and therefore the data were not corrected for outliers.

*2.10. Data Analysis*

The data obtained was analysed using statistical programme for social scientists (SPSS) to establish relationship between pesticide residue levels in the samples from different geographical location and seasons. Bivariate correlation coefficients were established using Pearson product moment correlation coefficient, "r", dimensionless index, whose value is in the range $-1.0 \leq r \leq 1.0$

**3. Results and Discussion**

The study which was conducted to determine the dissipation of carbendazim in two brands of French beans at different geographical locations and seasons showed the reduction of carbendazim residues in the beans with pods with time (Tables 1.3 and 1.4). There was variation in the initial carbendazim residue concentration at day zero (645.9mg/kg to 1260.7mg/kg) obtained for chariot 500 SC and Rodazim SC (825.7mg/kgto1039.4mg/kg) within and between seasons in the Kabaa site. The % degradation of carbendazim formulations showed insignificant variation regardless of the initial concentration. There was more than 96.7% reduction of carbendazim residues within 14 days after application of the pesticide (Tables 3 and 4).

| Short rains | | |
|---|---|---|
| | Mean Concentration (µg/kg) | |
| Days | Kabaa | Naivasha |
| 0 | 1260.7 | 970.3 |
| 3 | 496.2 | 467.3 |
| 7 | 220.1 | 188.5 |
| 14 | 17.7 | 31.6 |
| 16 | 5.7 | 0.7 |
| % Reduction by 3 day | 60.6 | 51.8 |
| % Reduction by 7 day | 82.5 | 80.6 |
| % Reduction by 14 day | 98.6 | 96.7 |
| % Reduction by 16 day | 99.5 | 99.9 |
| Long rains | | |
| | Mean Concentration (µg/kg) | |
| Days | Kabaa | Naivasha |
| 0 | 645.9 | 675.2 |
| 3 | 349.0 | 355.4 |
| 7 | 127.9 | 210.9 |
| 14 | 1.5 | 0.0 |
| 16 | 0.0 | 0.0 |
| % Reduction by 3 day | 46.0 | 49.5 |
| % Reduction by 7 day | 72.1 | 68.8 |
| % Reduction by 14 day | 99.8 | 100.0 |
| % Reduction by 16 day | 100.0 | 100.0 |

*Table 3: Degradation of Chariot 500SC in short and long rain seasons*

Results in Table 3 show that carbendazim pesticide residue levels, at 3 days after application were 496.2µg/kg and 349.0µg/k in Kabaa; 467.3µg/kg and 355.4µg/kg in Naivasha during the short and long rainy seasons respectively.

| Short rains | | |
|---|---|---|
| | Mean Concentration (µg/kg) | |
| Days | Kabaa | Naivasha |
| 0 | 1039.4 | 678.4 |
| 3 | 492.8 | 368.2 |
| 7 | 244.1 | 134.5 |
| 14 | 3.9 | 15.7 |
| 16 | 0.8 | 0.6 |
| % Reduction by 3 day | 52.6 | 45.7 |
| % Reduction by 7 day | 76.5 | 80.2 |
| % Reduction by 14 day | 99.6 | 97.7 |
| % Reduction by 16 day | 99.9 | 99.9 |
| Long rains | | |
| | Mean Concentration (µg/kg) | |
| Days | Kabaa | Naivasha |
| 0 | 825.7 | 905.5 |
| 3 | 455.8 | 576.3 |
| 7 | 205.8 | 192.8 |
| 14 | 0.0 | 0.0 |
| 16 | 0.0 | 0.0 |
| % Reduction by 3 day | 44.8 | 36.4 |
| % Reduction by 7 day | 75.1 | 78.7 |
| % Reduction by 14 day | 100.0 | 100.0 |
| % Reduction by 16 day | 100.0 | 100.0 |

*Table 4: Degradation of Rodazim SC in short and long rainsseason*

Results in Table 4 show that carbendazim pesticide residue levels, 3days after application of the pesticide were 492.8µg/kg and 455.8µg/k in Kabaa; 368.2µg/kg and 576.3µg/kg in Naivasha during the short and long rainy seasons respectively.

*3.1. Degradation trend of carbendazim in chariot 500 SC and Rodazim formulations*

The trends of dissipation of carbendazim for the two formulations used in the study during short and long rainy seasons were compared by plotting the averages of the determined concentration of carbendazim in the samples at Kabaa and Naivasha against time. There was a sharp decrease of carbendazim residue concentration in the first three days after application of the pesticides regardless of the season or geographical location in both chariot 500 SC (Figure 2) and Rodazim (Figure3). The determined concentration was below the maximum residue limit (200µg/kg) set by EU at 7 days (Figure 2) after application as shown in Figures 2 and 3.

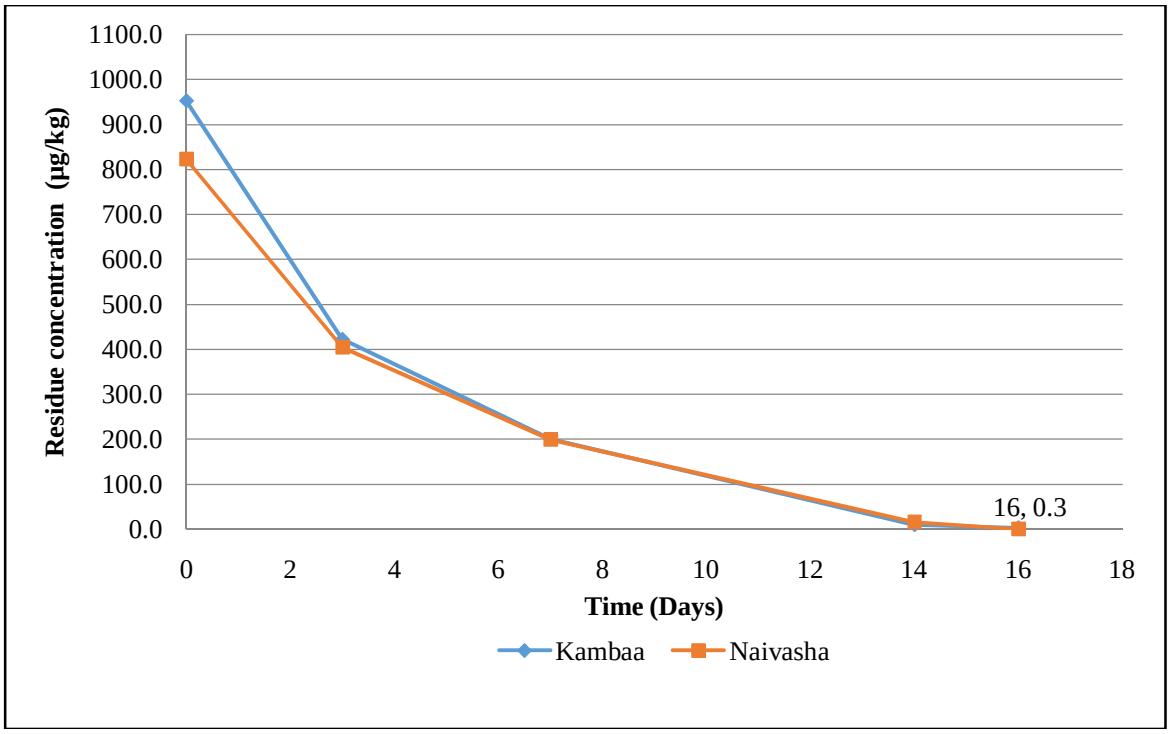


*Figure 2: Comparison of degradation of chariot 500SC at Kabaa and Naivasha in short and long rainy seasons*

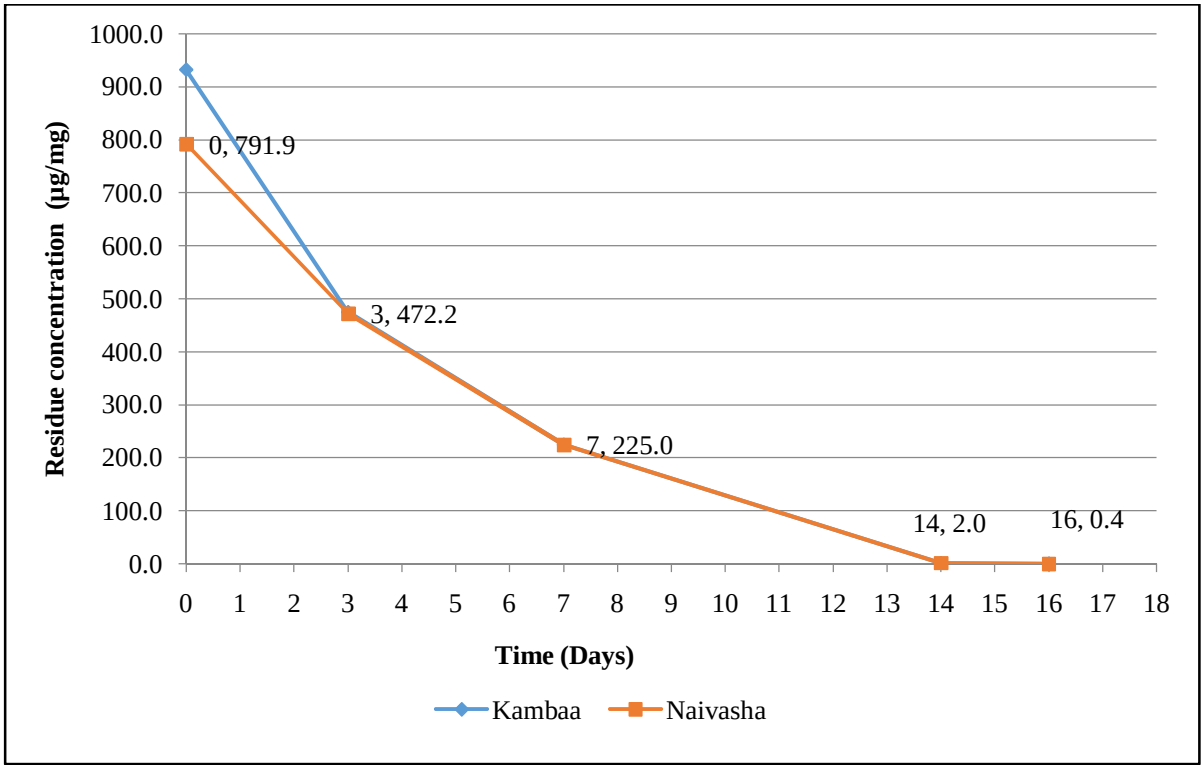


*Figure 3: Comparison of degradation of Rodazim at Kabaa and Naivasha during the short and long rainy seasons*

*3.2. Comparison of carbendazim degradation in Chariot 500 SC and Rodazim formulations*

Analysis of Variance (ANOVA) statistical tool in Microsoft excel was used to determine if there was correlation between breakdowns of carbendazim in French beans with the type of carbendazim formulation or geographical location. Two-way ANNOVA with replication was used to compare the breakdown of carbendazim based on pesticide formulation (Chariot 500 SC and Rodazim) and geographical location (Kabaa and Naivasha). The aim was to determine if there is statistical significance influence of carbendazim breakdown by the formulations or geographical location. The ANOVA analysis shows that the F calculated for formulation was 0.02 < 4.14 (F-critical) and F calculated for geographical location was 0.17 < 2.9 (F-critical), while at 95% confidence level there was no significant difference in carbendazim breakdown in the Serengeti French beans variety within the two formulations and geographical location. The output also shows that due to the interaction between the two variables (formulation and geographical location) the calculated value of F was 0.13 < 2.90 F-critical, an indication that there is no difference at 95% confidence. Therefore, it is concluded that there is correlation between the breakdown of carbendazim in French beans with formulation and geographical location.

The mean of the determined residue concentration was plotted against time. Figure 4 shows that there was rapid dissipation of carbendazim for the first 3 days after application. This could be attributed to the half life of the molecule in French beans.

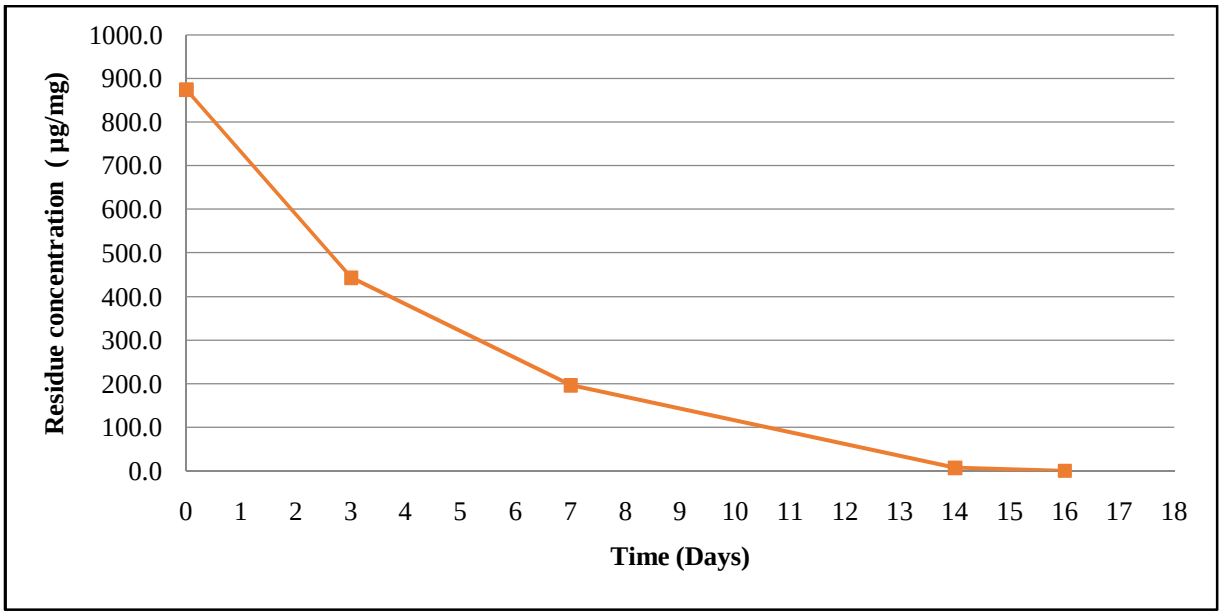


*Figure 4: Trends of carbendazim dissipation*

*3.3. Pre-harvest Interval for Carbendazim in French beans*

The half-life of carbendazim was used to calculate the PHI of carbendazim in French beans. Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Kar*et al.*, 2013) was used to obtain rate constant ($K_{obs}$) and half-life ($t_{1/2}$). Based on first order kinetic, a plot of negative ln average concentration of residues obtained from replicate samples taken at 0,3,7,14 and 16 days after application. $K_{obs}$ was obtained from the slope of the graph as 0.409 ($r^2$).

Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Kar et al., 2013) to obtain rate constant ($K_{obs}$) and half-life ($t_{1/2}$).

$$r = dC/dt = kKC/(1+KC) \quad \ldots\ldots(1)$$

Consider the half-life of the reaction where the remaining concentration of the pesticide is half the original amount; Ct= Co/2 and substituting in equation (1)

$$\ln(Co/2Co) = Kt_{1/2} \quad \ldots\ldots(2)$$

$$\ln 0.5 = -Kt_{1/2}. \quad \ldots\ldots(3)$$

$$-0.693/K = t_{1/2} \quad \ldots\ldots(4)$$

In this study the degradation of carbendazim follows Langmuir-Hinshelwood kinetic equation and using equation (4) the half-life of carbendazim in French beans was found to be 1.7 days. Chariot 500SC and Rodazim formulation of carbendazim showed a similar pattern of degradation in short and long rain seasons in Kabaa and Naivasha sites. The EU set MRLS for French beans (beans with pods) according (EU) regulation 369/2005 is set at 200µg/kg. The pre-harvest interval (PHI) of carbendazim was calculated at 50% of the EU set MRLs using equation ($InC_t = InC_0 - K_{obs}X_t$). Where $C_t$ is the calculated concentration at 50% of MRLs for French beans (100µg/kg), $C_0$ is the average deposition of carbendazim obtained at time zero in the study and $K_{obs}$ is the gradient obtained from the regression curve for disappearance of carbendazim. The PHI for carbendazim in French beans was found to be 5.4 days (200µg/kg) after application. This indicated that pesticide residue on or in French beans treated with carbendazim at a rate of 6.25 kg per hectare for the control of fungal diseases and following good agricultural practice (GAP) will be below the EU legally accepted MRLs of 200µg/kg 5 days after application.

**4. Conclusions**

The study revealed that, degradation of carbendazin pesticide in chariot 500SC and Rodazim formulations in French beans is not significantly different. The degradation trends were comparable in short and long rains seasons. There was significant degradation of more than 50% of carbendazin in the first 3 day after application and more 95% in the second week. The study established that 5 days after application of carbendazim according to the right dosage and technique is long enough to reduce the pesticide residue below the EU legally accepted MRLs of 200µg/kg in French beans. A 5day PHI will reduce the level of human and animal exposure to carbendazim which increases the level of compliance, competitiveness of Kenyan produce and the market access.

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