

Nutrition Management in Organic Vineyards

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Executive summary

Nutrition management in organic vineyards is a big challenge due to limitations in availability and usage of fewer products, of organic origin. Viticulture and winery waste & by-products like vine prunings, grape stalks, grape pomace, lees, lees filter cakes and cellulose filter pads (Organic wastes) contain enormous amounts of nutrients which could be adequately recycled in the vineyards. Present study was aimed to recycle such decomposed organic wastes (OW) mixed with other organic products like cow manure (CM) and saw dust (SD) designated as organic mixtures in following ratio:

Mixture 1 (M1): 1(CM): 1(OW): 1(SD)

Mixture 2 (M2): 1(CM): 1(OW): 2(SD)

Both organic mixtures had comparable pH values with major differences in higher electrical conductivity of Mixture 1 due to increased ammonia nitrogen and total Kjeldahl nitrogen levels than Mixture-2. However, the carbon/nitrogen ratio was higher in Mixture-2. The experiment was conducted in Randomized Block design having 2 by 2 factorial design {application of two organic mixtures (1&2) in relation to two types of dose quantities (D1=1.0kg and D2=2.0 kg/plant)} using five wine grape varieties viz. Chardonnay, Gewurztraminer, Pinot Gris, Riesling (white varieties) and Zweigelt (red variety). Two doses of each mixture were applied (before cane formation and prior to veraison) excluding controls during study to observe the qualitative and quantitative effects on growth characteristics, crop quantity and grape juice quality. Plants received with organic mixture 1 (dose 1 or 2) were observed slightly better than mixture 2 in terms of rapid cane growth, darker green leaves, dense canopy. There was no nutrient deficiency or toxicity observed in any of the treatments. The statistical results using Analysis of Variance (ANOVA) methodology indicated wide range of variation in data for the bud-break frequency, cane formation, stem diameter, cluster weight and berry size. None of the treatment combinations stood apart from the others except differences across the varieties. It could be partly due to the fact that, initial nutrients release from composts is usually quite slow which increases with time.

The chemical analyses of juice for pH, Brix, Titrable acidity (TA), Total phenols (TP) and Yeast Assimilable Nitrogen (YAN) gave interesting results. Chardonnay, Gewurztraminer, Riesling and Zweigelt showed increased pH responses to both the mixtures (M1 and M2) and doses (1 and 2), whereas Pinot Gris had comparable pH values. The sugar contents (Brix) were slightly higher for Gewurztraminer and Riesling on both mixtures and doses than Chardonnay, Zweigelt and Pinot Gris were comparable with their controls. Titrable acidity values were higher with Mixture 1 and dose 1 for all the varieties. There was an increasing trend of total phenols with both doses and mixtures for all varieties. Higher phenol contents imparted deep pigmentation to the berries and juice coloration. YAN values were recorded higher for Gewurztraminer, Zweigelt and Riesling than Chardonnay and Pinot Gris. Overall Mixture 1 with Dose 1 was best.

Results of this study indicate that organic viticulture and winery wastes could be successfully recycled in the vineyards to improve the soil organic matter, grape juice quality and partial improvement in plant vigor and crop quantity in the first year which is expected to increase in subsequent years. Additionally, our experiment shows the potential for further researches on nutrition management and sustainability of organic vineyards.

Introduction

In 2009, Canada's organic wine grape production was 320 acres (Certified Organic Production Statistics for Canada 2009). In the last few years, Canadian organic viticulture industry has grown at a rapid pace. British Columbia is the major wine grape producing province followed by Ontario. At present, organic viticulture industry is faced with the major challenge of nutrition management in vineyards to produce best quality grapes for wines production. However, goal has been also to develop vineyards that are sustainable and harmonious with the environment. The major limitations for most organic vineyards are availability and usage of a fewer products as nutrients, of organic origin (Anonymous, 2002). Grape growing and wine making process generate a number of organic wastes and by-products viz. vine prunings, grape stalks, grape pomace, lees, lees filter cakes and cellulose filter pads. These products contain enormous amounts of NPK and other nutrients (Seenappa, 2012; Westover, 2006), which need to be recycled safely within the vineyards. Therefore, sustainability of organic vineyards is foremost importance. There are few reports available on recycling of viticulture, winery waste & by products and its potential applications (Arvanitoyannis *et al.*, 2006; Bertran *et al.*, 2004; Ferrer, 2001; Nerantzis *et al.*, 2006). Present study was undertaken with the following objectives:

1. To recycle various viticulture and wine making wastes & by-products in the organic vineyards upon mixing them with other organic products (cow manure and saw dust)
2. To explore their effects on improvement in grape vine vigor, yield and juice quality

Materials and methods

1. **Site description:** Experiments were conducted in Block 'B' (7.5 acres), Kalala Organic Vineyards at West Kelowna, BC. At Kalala commercial vineyards, grapevine varieties were marked with variety name and number. Each row usually had one type of variety. The plant growth (shoots/canes) was managed vertically and horizontally with the support of metal wires fixed at different heights between the wooden posts. The distance between two wooden posts was 20 feet with a planting density of five plants (4 feet apart) in between.
2. **Selection of wine grape varieties:** In the vineyard, plants of same age and vigor were selected for each experimental variety. The five commercially important wine grape varieties selected for present study were:
 - Chardonnay: White variety, maturing mid-late season, moderate vigour
 - Gewürztraminer: White variety, maturing early in season, moderate vigour
 - Pinot Gris: White variety, maturing mid-late season, moderate vigour
 - Riesling: White variety, maturing late season, vigour moderate to high.
 - Zweigelt: Red variety, late season maturity, highly vigorous.
3. **Pruning of experimental varieties:** A combined type of pruning 'Cane and Spur' was manually done to all the experimental plants before initial bud break. During pruning, on one side of the trunk the cordon was removed leaving only one healthy cane (8-10 buds) originated from trunk while on the other side the canes on old cordon were pruned back to spurs. Each plant had approximately 8-10 spurs, each spur having 3 good buds (Figure 1: 1).

- Preparation organic mixtures:** Last year's viticulture and winery wastes & by products known as organic wastes (OW) like pomace, grape stalks, lees, lees filter cakes, cellulose filter pads, prunings were transported to a composting site of vineyard, subsequently crushed to powder/granules prior to mixing with other organic products viz. rotten cow manure (CM) and sawdust (SD). Organic products were mixed in different ratio to prepare following organic mixtures:

Mixture 1: 1(CM): 1(OW): 1(SD)

Mixture 2: 1(CM): 1(OW): 2(SD)

Organic mixtures were analysed at Exova, Surrey, BC (www.exova.com) for NPK and microelements levels (Annexure 1). The analysis report indicated that both organic mixtures had comparable pH values with major differences in electrical conductivity (Mixture 1=3.87 mS/cm and Mixture 2=2.04 mS/cm). This difference could be due to higher ammonia nitrogen (N-NH₃) in Mixture -1. The total Kjeldahl nitrogen was also higher in Mixture-1 (1.14%) than Mixture-2 (0.44%). However, the carbon/nitrogen ration was higher in Mixture-2 (35.7%) possibly due to double quantity of saw dust.

- Experimental design:** Experimental study was conducted in Randomized Block design having 2 by 2 factorial design {application of two organic mixtures (1&2) in relation to two types of dose quantities (1.0 and 2.0 kg/plant)}.
- Experimental plan:** Twenty five plants of each variety in vineyard rows were marked individually with treatment codes (Figure 1: 2) for various organic mixtures applications. The experimental plot size was taken 25 plants per variety, due to easy record of observations for 125 plants (25 plants X 5 varieties) in a day for each growth parameter to avoid time gaps in data record. There were five replicates for each variety. Each replicate had five plants including a control. The control plants were not applied with any organic mixture. Four plants out of five were randomly selected to receive the four mixture treatments. This random allocation was repeated for each block of 5 plants to satisfy the statistical requirement of Randomized Block design protocol.
- Soil application of organic mixtures (A & B individually) in vineyards:** All plants in a replicate (excluding control) for each variety were applied (Figure 1: 2) different quantities {1.0 kg/plant (= 1.0 tons/acre) and 2.0 kg/plant (2.0 tons/acre)} of 'organic mixtures' two times during the entire course of study.

First dose of each organic mix was applied per plant before cane formation (May 20, 2012): 1.0 kg/plant (= 1.0 tons/acre) and 2.0 kg/plant (=2.0 tons/acre)

Second dose of each organic was applied per plant before Veraison (July 26, 1012): 1.0 kg/plant (= 1.0 tons/acre) and 2.0 kg/plant (= 2.0 tons/acre)

- Statistical analysis of data:** The data for plant vigour and crop quantity were analysed using a standard statistical software package such as JMPIN (www.jmp.com). Detailed statistical analysis such as Multivariate Analysis of Variance (MANOVA) would reveal the efficacy of the two organic mixtures and the two dose levels with respect to various plant growth characteristics. Since the treatments were being tested for various grape varieties at the same time, we could get a clear picture of which mixture-dose

combinations were beneficial for which varieties. Statistical analysis for data obtained on chemical properties of grape juice was not considered, to be done.

9. **Irrigation management and other organic nutrients application:** In a growing season (May 15, 2012 to October 30, 2012), a generalised schedule of overhead irrigation, drip and foliar nutrition was followed at the experimental site. Considering rainy weather conditions in May and June months, overhead irrigation was avoided. Over head irrigation using sprinklers was provided only two times in the summer (July 7 and July 28, 2012), each six hours to moisten the soil conditions for root growth, nutrient uptake and washing off powdery mildew spores from canopy. In the later months, the over head irrigation was stopped to prevent the rotting of developing grape clusters. The drip system was the main source of irrigation and effective application of various organic nutrients to the root zone of vines, besides other foliar sprays taken with boom sprayer. Drip irrigation frequency, depending upon the rainfall and soil moisture conditions was generally provided once in a month (May 2012 to October 2012) for 16-20 hrs contained with or without organic nutrients. Foliar sprays of organic nutrients were taken intermittently with drip irrigation cycles.

Table 1: Drip application of organic nutrients at experimental site

| Organic products | Rate per acre/season | Quantity applied/season |
|-------------------------|-----------------------------|--------------------------------|
| Kelp grow | 1.77 lit | 13.27 lit |
| Blood meal | 28.5 kg | 213.75kg |

Table 2: Foliar sprays/application of organic nutrients at experimental site

| Organic products | Rate per acre/season | Quantity applied/season |
|-------------------------|-----------------------------|--------------------------------|
| Sulphur | 3.5 kg | 26.25 kg |
| E-13 oil | 3.8 lit | 28.50 lit |
| Kelp grow | 2.0 lit | 15.00 lit |
| Blood meal | 7.0 kg | 52.50 kg |
| Calcium chloride | 333.3 gm | 2.50 kg |
| Solubor | 133.33 gm | 1.00 kg |

10. Quantitative and qualitative measurements of growth characteristics in vineyards

- i) **Plant vigor** – Data on bud break frequency (total buds), cane formation and maturation, stem diameter were recorded for each experimental treatment. Bud break frequency was calculated by counting total number of buds sprouted in beginning of growing season (May third week, 2012) out of available number of dormant buds on canes and spurs. After the initial bud break, a bud thinning practice was employed in May 4th week to leave only desired number healthy buds

- (Figure 1: 3) on each spur or cane to develop into new shoots or new canes. Cane formation was recorded by counting available number of new canes developed on cane or spur pruned sides of a plant. One cane per treatment, at 30 cm height from its origin, was randomly (left to right) measured (in mm) with Vernier Calipers (make Performance Tool) for stem/cane diameter. Cane diameter data were recorded in August 2012 end/September 2012 beginning, upon development of grape clusters.
- ii) **Crop quantity** – The data for each treatment were recorded on flowering frequency, veraison, cluster shape, cluster type, cluster weight, number of clusters/vine, berry shape and berry size. A flowering frequency of more than 75% flowers successfully self-fertilised and developed into berries on each plant was considered as uniform flowering pattern (Figure 1: 4) whereas less than 75% was non-uniform. Flowering and fruiting was coupled with rapid growth of canes bearing healthy green leaves (Figure 1: 5). Leaf thinning practice was performed by removing lower 3-4 leaves on canes to expose the developing clusters (Figure 1: 6) for proper development of berries and achieving veraison. In some of the cases unwanted clusters from canes were removed (cluster thinning) leaving only 1-2 clusters per cane. Veraison, often expressed as onset of ripening, was evaluated as uniform and non-uniform based on desired berry pigment development, softening of berry skins due increase in sugar content and decreased acids. Observations on cluster shape & type, berry shape were recorded as per the defined standards for each variety. A special emphasis was given to observe abnormal development of cluster or berries due application of organic mixtures. One cluster per treatment and one berry per cluster were randomly selected for measurement of cluster weight (in gm) and berry size (in mm). The clusters were weighed fresh on a scientific scale while berry sizes were measured by Vernier Calipers.
- iii) **Crop health**- A routine spray schedule (May 15, 2012 to August 15, 2012) of Sulphur (Kumulus™) every 15-20 days (@ 1.7kg/acre), depending upon the weather conditions was followed at experimental site for prevention of powdery mildew on foliage, stems and clusters. All treatments plants were monitored weekly for occurrence, type and frequency of any diseases or pests during entire course of study consulting 'Best practices guide for Grapes for British Columbia Growers' (Anonymous, 2010).
11. **Chemical analysis of grape juice:** One cluster from each replicate per treatment was harvested randomly (from left to right on a plant). Thus, five clusters for each treatment were crushed to obtain sufficient quantity of juice. Juice for all varieties and treatments were collected in glass bottles having screw caps, stored in fridge (at 4⁰C) to prevent any subsequent natural fermentation affecting the chemical properties. Following chemical analyses were carried out:
- i) **pH:** pH readings were measured by dipping a pH meter (Tracer, Make LaMotte) in the juice as described in 'Analysis of grapes and wine: technique and concepts' (Patrick Iland, Nick Bruer, Greg Edwards, Sue Weeks and Eric Wilkes, 2004)

- ii) **Brix (sugar):** The available sugars in the grape juice, expressed in °Brix, was measured using a hand held Refractometer (Make, Bosa). Refractometer measures the refractive index of a solution i.e. what extent a beam of light passing through the solution is bent.
- iii) **Titrateable acidity:** Titrable (TA) measures the concentration of all the available hydrogen ions present in the sample juice or wine. TA was analysed using the method described in 'Analysis of grapes and wine: technique and concepts' (Patrick Iland, Nick Bruer, Greg Edwards, Sue Weeks and Eric Wilkes, 2004)
- iv) **Total phenols:** Total phenols test were conducted using Erbsloh-Easy Lab (www.erbsloh.com), strips based on Folin Ceocalteau method.
- v) **Yeast assimilable nitrogen (YAN):** YAN was analysed using Megazyme™ (Denmark) enzymatic colorimetric method at Analytical Juice and Wine Laboratory, Kelowna, BC (www.winelaboratory.com).

Results

We start with some graphical displays to have a visual feel of the results.

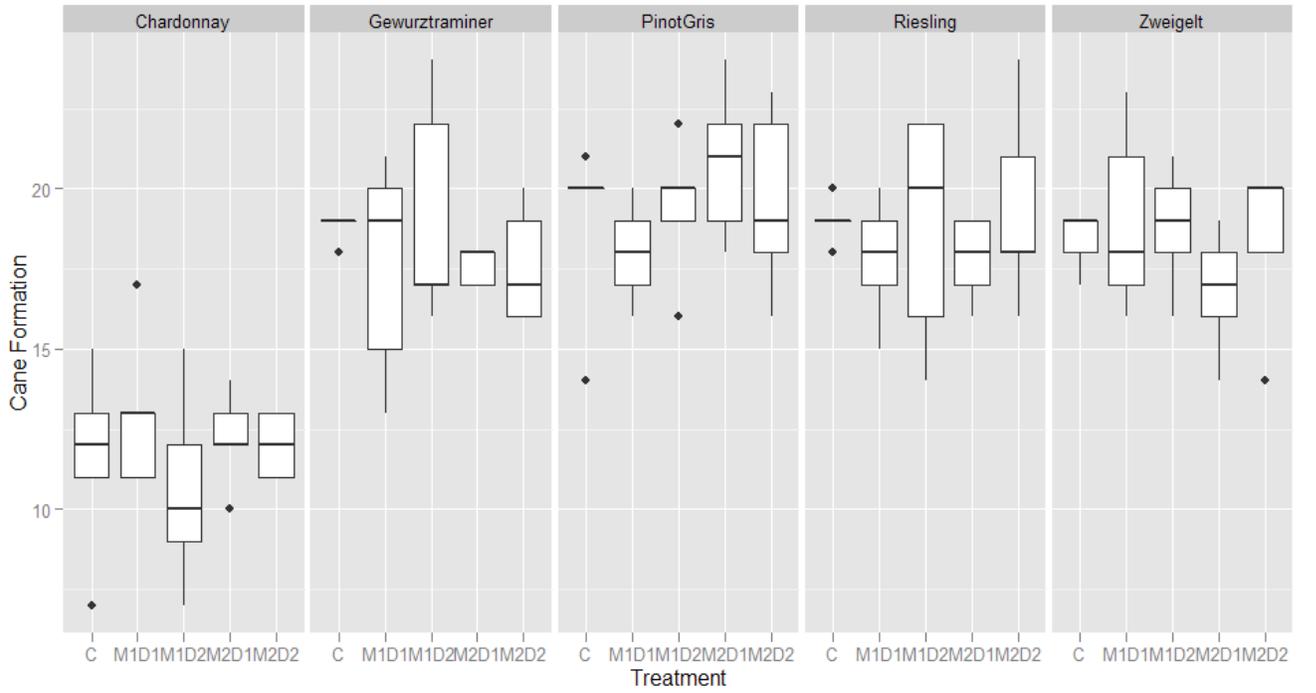
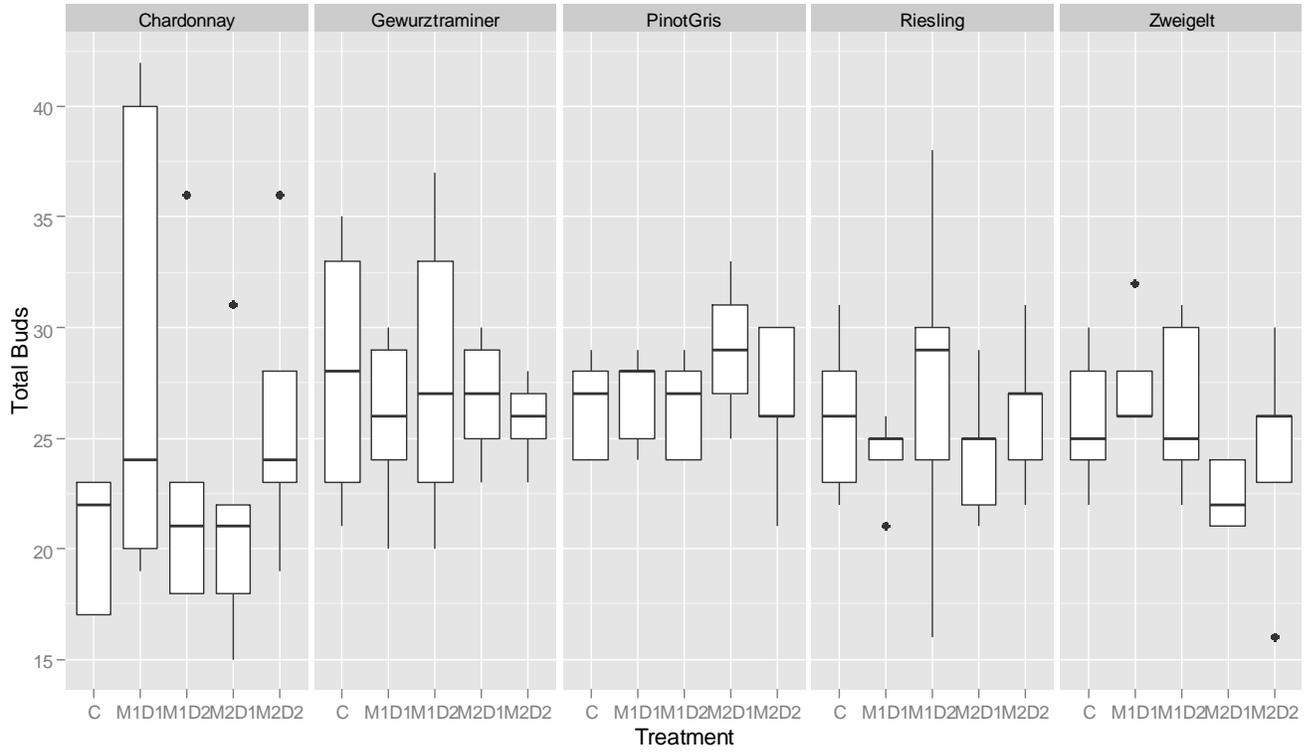
Boxplots: To facilitate the comparison of five treatment combinations (four test treatments and control C) across the five varieties, boxplots of individual data values for some selected characteristics are included. The vertical height of the boxplot gives an idea of the variability of the responses under each treatment combination. The horizontal line inside the box represents the median (average) value of the response.

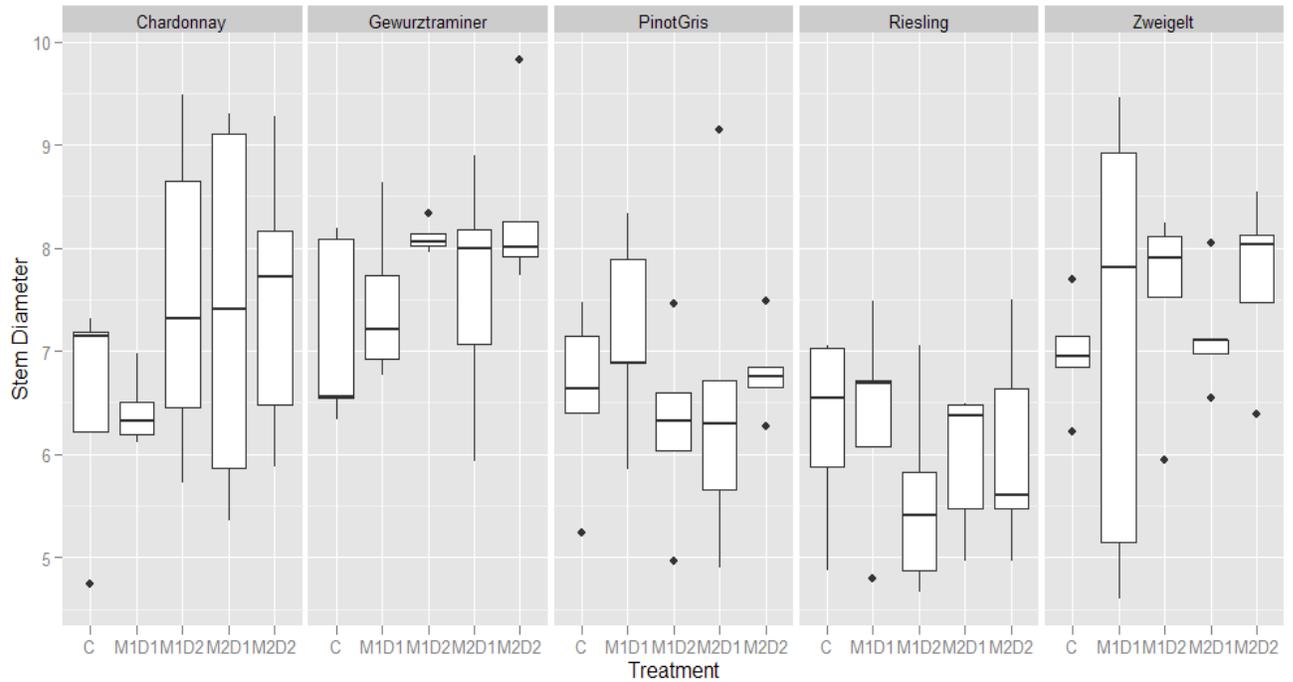
The boxplots are grouped together by variety arranged from left to right as: Chardonnay, Gewurztraminer, Pinot Gris, Riesling and Zweigelt. Within each variety, the treatment combinations appear from left to right as: Control, M1D1, M1D2, M2D1 and M2D2.

1) Quantitative and qualitative measurements of growth characteristics in vineyards:

- i) **Plant vigor:** During initial bud break phase, all treatments including control exhibited similar responses for all the varieties. However, in the later phase of cane growth and canopy development there were significant morphological differences among treatments. The leaves of mixture treated plants were relatively darker green and slightly bigger than their controls. The cane development was also faster achieving higher lengths as compared with their controls, which was managed by pruning off the extra top growth. There were no symptoms of nutrient toxicity and /or deficiencies observed on foliage in all varieties and treatments. Overall, the mixture treated plants were qualitatively better than their respective controls. The general trend of plant vigor observed was, Zweigelt > Chardonnay > Pinot Gris > Riesling > Gewurztraminer.

The boxplots for the bud-break frequency, cane formation and stem diameter reveal a wide range of variation in the data when we compare the five treatments for each of the varieties. None of the treatment combinations stand apart from the others.





Observation: Apart from the differences across the varieties (which is expected), we can't say that the treatment combinations produced different results for the plant vigor.

Comparison of Mean Response Values: The following tables and bar charts compare the mean values as computed from 5 replicate plants for each treatment combination for Bud-break frequency, cane formation and stem diameter.

Table 3: Mean values of Total buds (bud break frequencies) of varieties X treatments

| Total Buds | | | | | |
|------------|------|------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 20.4 | 28.0 | 26.4 | 26.0 | 25.8 |
| M1 D1 | 29.0 | 25.8 | 26.8 | 24.2 | 27.6 |
| M1 D2 | 23.2 | 28.0 | 26.4 | 27.4 | 26.4 |
| M2 D1 | 21.4 | 26.8 | 29.0 | 24.4 | 22.4 |
| M2 D2 | 26.0 | 25.8 | 26.6 | 26.2 | 24.2 |
| | | | | | |

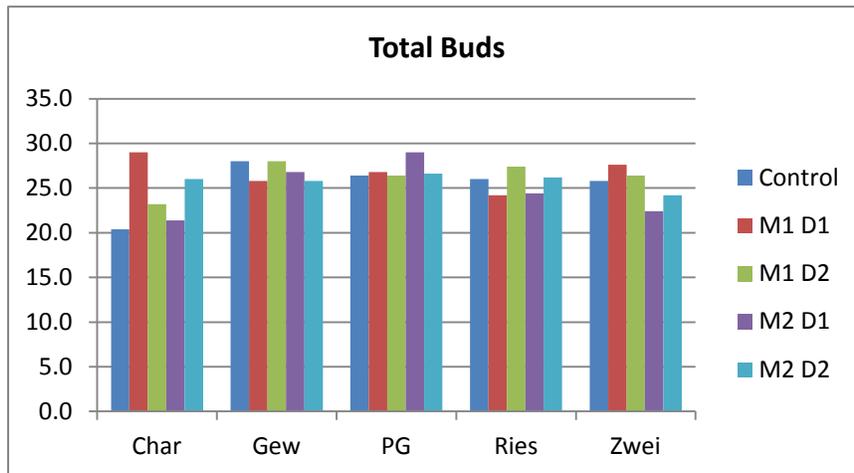


Table 4: Mean values of cane formation of different varieties X treatments

| Cane Formation | | | | | |
|----------------|------|------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 11.6 | 18.8 | 19.0 | 19.0 | 18.4 |
| M1 D1 | 13.0 | 17.6 | 18.0 | 17.8 | 19.0 |
| M1 D2 | 10.6 | 19.2 | 19.4 | 18.8 | 18.8 |
| M2 D1 | 12.2 | 17.6 | 20.8 | 17.8 | 16.8 |
| M2 D2 | 12.0 | 17.6 | 19.6 | 19.4 | 18.4 |

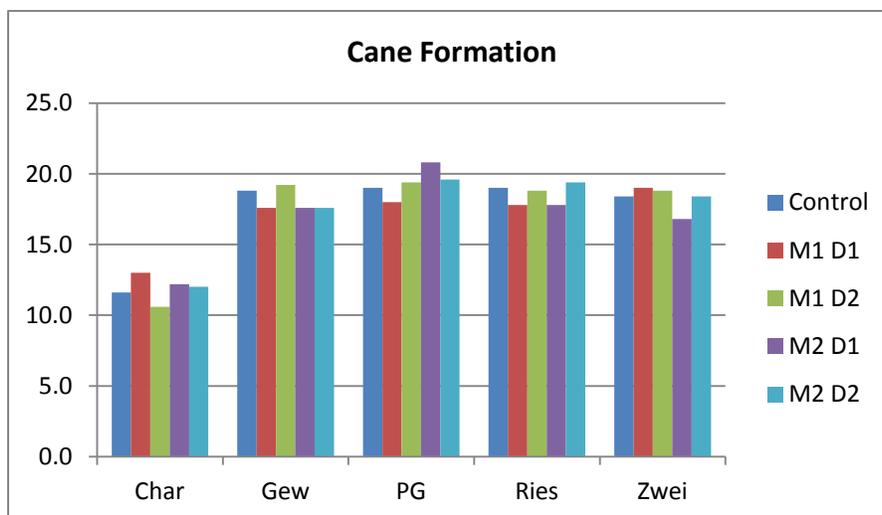
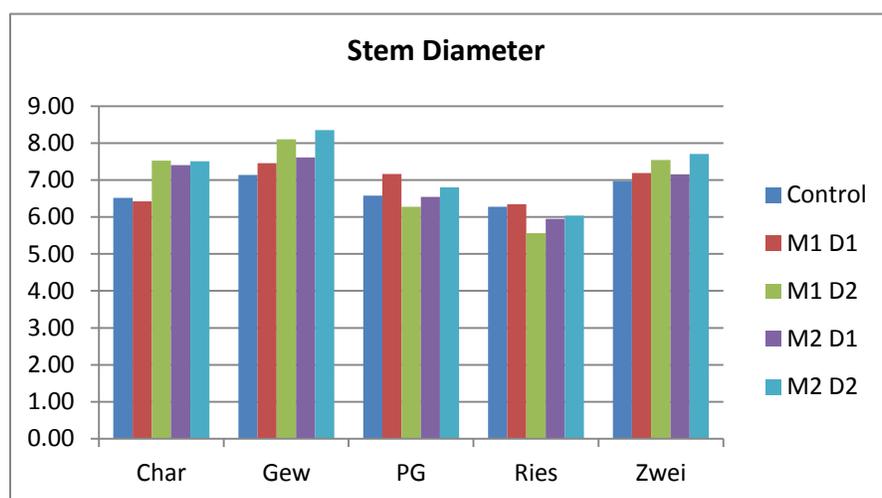


Table 5: Mean values of stem diameter of different varieties X treatments

| Stem Diameter | | | | | |
|---------------|------|------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 6.52 | 7.14 | 6.58 | 6.28 | 6.97 |
| M1 D1 | 6.42 | 7.46 | 7.17 | 6.35 | 7.19 |
| M1 D2 | 7.53 | 8.10 | 6.27 | 5.56 | 7.54 |
| M2 D1 | 7.40 | 7.61 | 6.54 | 5.95 | 7.15 |
| M2 D2 | 7.51 | 8.35 | 6.80 | 6.03 | 7.71 |



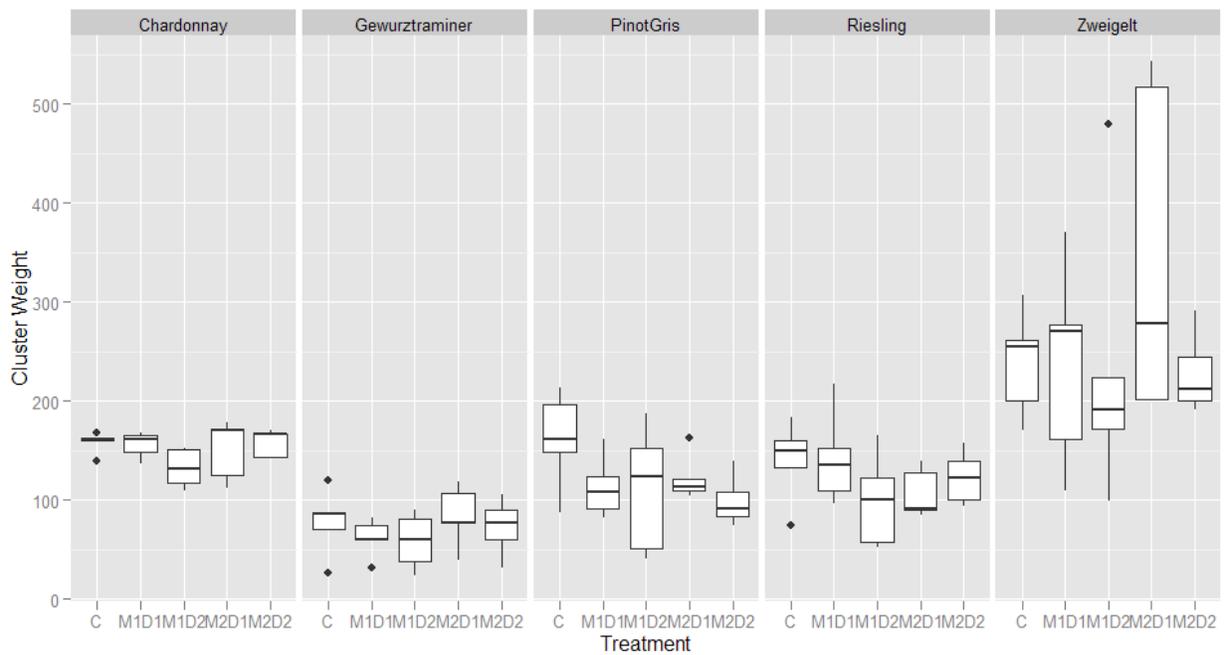
ii) Crop quantity –

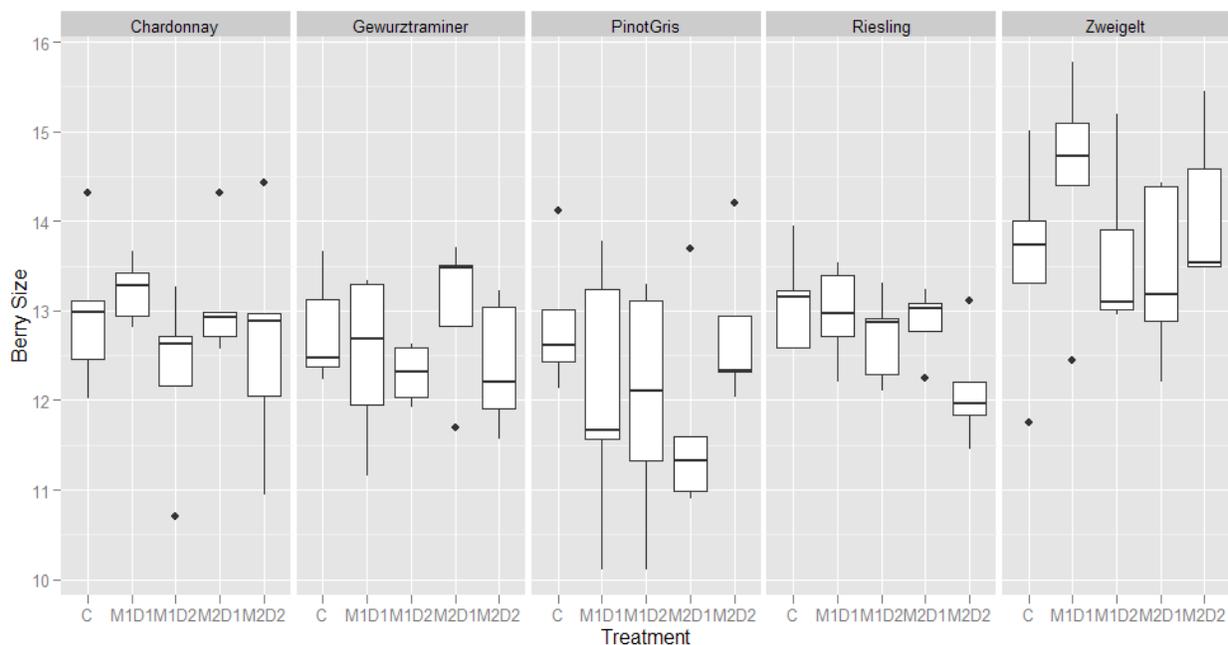
A uniform flowering pattern (>75%) was observed for all the treatments including controls for all the selected varieties. There was no abnormal effect of mixtures on any of the treatments. However, flowering and berry formation was observed sooner on spur pruned side of the plant than cane pruned. The process of berry formation had significant differences among the varieties but comparable in all treatments including controls. The berry development response was best in Zweigelt, Pinot Gris, Chardonnay followed by Riesling and Gewurztraminer. All treatments plants and controls exhibited normal veraison. Riesling showed late cap fall in flowers on spur side of pruning as compared to cane side. Interestingly, varieties viz. Zweigelt, Pinot Gris and Gewürztraminer, Mixture 2 (dose 1 and 2) treated plants developed darker berries than Mixture-1 treated plants. On the outset of veraison, seeds of all varieties Controls attained brown color and tasted more sugary as a sign of definite maturity while same time the all other treatments had partially green seeds and less sweeter. This could possibly be due to continued vegetative growth by uptake of additional nutrients supplied through the mixtures, delaying the berry maturity. On crop maturity, there were no abnormal differences observed in cluster type, cluster shape and berry shape among treatments except varietal differences (Figure 2). The general observations on crop maturity of experimental plants are mentioned in Table 6.

Table 6: Qualitative characteristics on crop maturity

| Varieties | Cluster type | Cluster shape | Berry shape |
|----------------|----------------|--------------------|-------------|
| Chardonnay | Compact | Long conical | Round |
| Gewürztraminer | Mostly compact | Short conical | Round |
| Pinot Gris | Compact | Short conical | Round |
| Riesling | Compact | Short conical | Round |
| Zweigelt | Compact | Conical shouldered | Round |

Comparing boxplots for the cluster weight and berry size data, we noticed again significant differences (nothing surprising) amongst the five varieties. But within each variety, none of the treatment combinations stood out from the others in showing effect on the cluster weight or on the berry size.





Observation: Apart from the differences across the varieties (which is expected), we can't say that the treatment combinations produced different results for the crop quantity.

Comparison of Mean Response Values: The following tables and bar charts compare the mean values as computed from 5 replicate plants for each treatment combination for Cluster weight and berry size.

Table 7: Mean values of cluster weight of different varieties X treatments

| Cluster Weight | | | | | |
|----------------|-------|-------|-------|-------|-------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 158.4 | 78.2 | 161.6 | 140.4 | 238.8 |
| M1 D1 | 155.8 | 61.6 | 113.2 | 142.4 | 237.4 |
| M1 D2 | 132.6 | 58.75 | 111.2 | 99.6 | 233.4 |
| M2 D1 | 151.6 | 84.0 | 122.2 | 106.8 | 348.2 |
| M2 D2 | 158 | 72.9 | 99.4 | 123 | 227.8 |

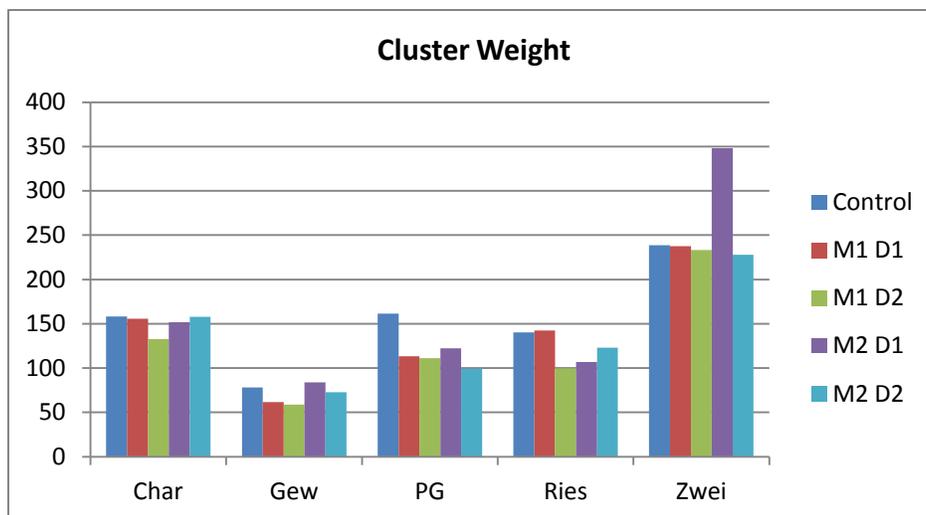
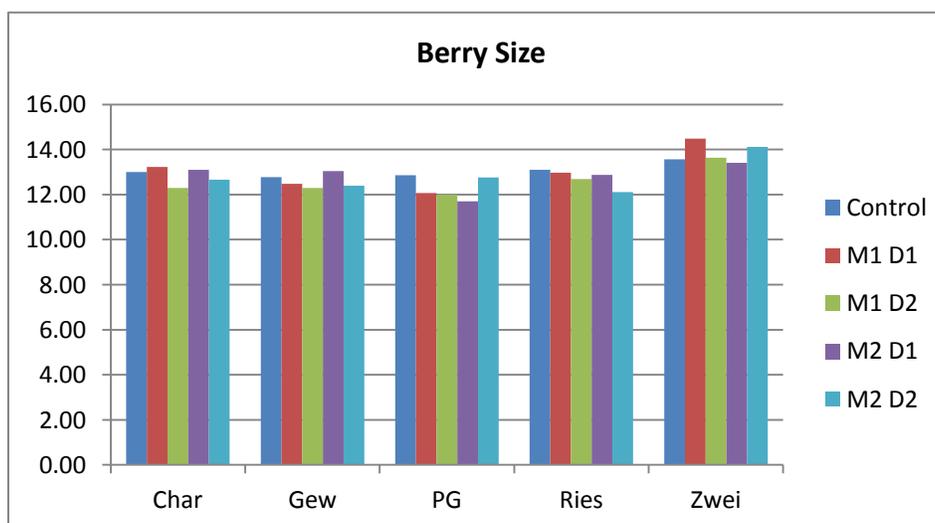


Table 8: Mean values of berry size of different varieties X treatments

| Berry Size | | | | | |
|------------|-------|-------|-------|-------|-------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 13.00 | 12.77 | 12.86 | 13.10 | 13.56 |
| M1 D1 | 13.22 | 12.48 | 12.07 | 12.97 | 14.48 |
| M1 D2 | 12.29 | 12.30 | 11.99 | 12.70 | 13.63 |
| M2 D1 | 13.10 | 13.05 | 11.70 | 12.87 | 13.42 |
| M2 D2 | 12.66 | 12.39 | 12.76 | 12.11 | 14.11 |



Formal statistical analysis was done by the Analysis of Variance (ANOVA) methodology using the JMPIN statistical software package. ANOVA technique was applied for each characteristic separately. The ANOVA F-test is used as the first step to see if the treatment combinations differ from one-another in affecting the response characteristic. In case the F-test shows statistically significant (say at 5% level) effects, further analysis is carried out to identify which treatment combinations results in higher (or lower) average response.

The following table (Table 9) shows the F-ratio values for a series of ANOVA tests done for response data on Bud-break frequency, cane formation, stem diameter, cluster weight and berry size. All the F-ratio values for the testing the treatment effects show non-significant values. That is, treating the grapevines with any of the four mixture treatments does not affect the plant vigor and the crop quantity.

Table 9: Showing F-ratio values for various quantitative data on plant vigour and crop maturity:

| Analysis of Variance Summary | | | | | | |
|------------------------------|------------------|---------|----------------|---------|--------------------------------------|---------|
| Response | Treatment Effect | | Variety Effect | | Treatment*Variety Interaction Effect | |
| | F-ratio | p-value | F-ratio | p-value | F-ratio | p-value |
| Total Buds | 0.532 | 0.712 | 1.479 | 0.214 | 0.989 | 0.474 |
| Can Formation | 0.133 | 0.97 | 43.371 | <0.0001 | 0.764 | 0.722 |
| Stem Diameter | 0.943 | 0.442 | 8.828 | <0.0001 | 0.668 | 0.819 |
| Cluster Weight | 1.438 | 0.227 | 33.572 | <0.0001 | 0.979 | 0.485 |
| Berry Size | 1.156 | 0.335 | 10.668 | <0.0001 | 1.001 | 0.463 |

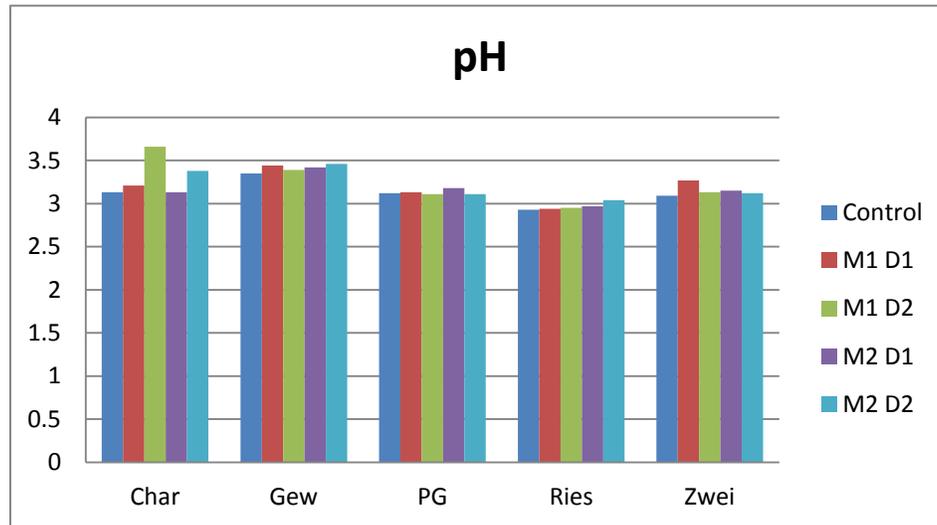
iii) **Crop health:** There was no significant occurrence of powdery mildew disease or any other insect/fungal pest was observed during the entire course of study. In general variety Riesling was observed relatively susceptible to powdery mildew disease which was successfully controlled by periodic sprays of Sulphur (Kumulus™).

2) Chemical analysis of grape juice:

i) **pH:** The pH values for Chardonnay, Gewurztraminer, Riesling and Zweigelt for both the mixtures and doses were higher than their respective controls (Table 10). Double dose (2.0kg) increased pH in grapes, than single dose (1.0kg). However, in case of Pinot Gris the pH values of treatments and controls were comparable.

Table 10: pH analysis of different varieties X treatments

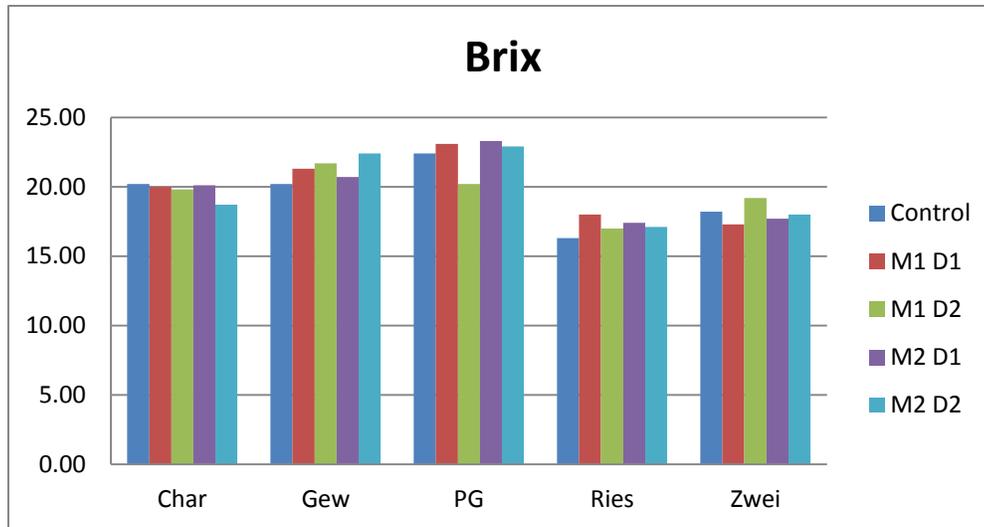
| pH | | | | | |
|-----------|------|------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 3.13 | 3.35 | 3.12 | 2.93 | 3.09 |
| M1 D1 | 3.21 | 3.44 | 3.13 | 2.94 | 3.27 |
| M1 D2 | 3.66 | 3.39 | 3.11 | 2.95 | 3.13 |
| M2 D1 | 3.13 | 3.42 | 3.18 | 2.97 | 3.15 |
| M2 D2 | 3.38 | 3.46 | 3.11 | 3.04 | 3.12 |



- ii) **Brix (sugar):** Gewürztraminer and Riesling mixtures (1 and 2) treated plants showed slight improvement in brix as compared with their controls (Table 11). There was no significant sugar content increase observed in Chardonnay, Zweigelt and Pinot Gris for treatments as compared with their controls.

Table 11: Brix (⁰B) analysis of different varieties X treatments

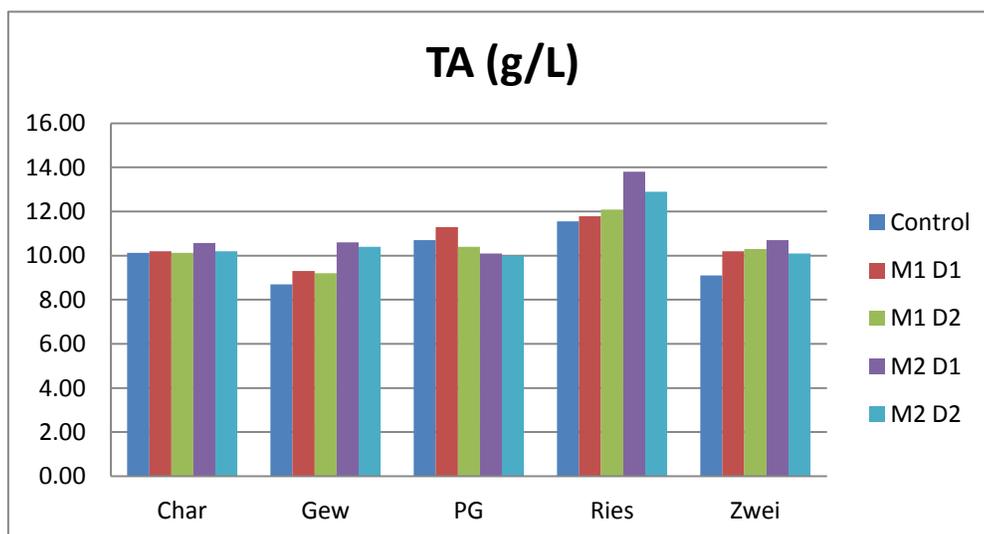
| Brix (⁰ B) | | | | | |
|------------------------|------|------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 20.2 | 20.2 | 22.4 | 16.3 | 18.2 |
| M1 D1 | 20.0 | 21.3 | 23.1 | 18.0 | 17.3 |
| M1 D2 | 19.8 | 21.7 | 20.2 | 17.0 | 19.2 |
| M2 D1 | 20.1 | 20.7 | 23.3 | 17.4 | 17.7 |
| M2 D2 | 18.7 | 22.4 | 22.9 | 17.1 | 18.0 |



iii) **Titration acidity (TA):** Chardonnay, Gewurztraminer, Riesling and Zweigelt treatments showed increase in TA for both the mixtures at single dose (1.0kg/plant) as compared to their Controls (Table 12). However for Pinot Gris treatment M1D1 was the recorded best (11.3 g/l).

Table 12: Titration acidity analysis of different varieties X treatments

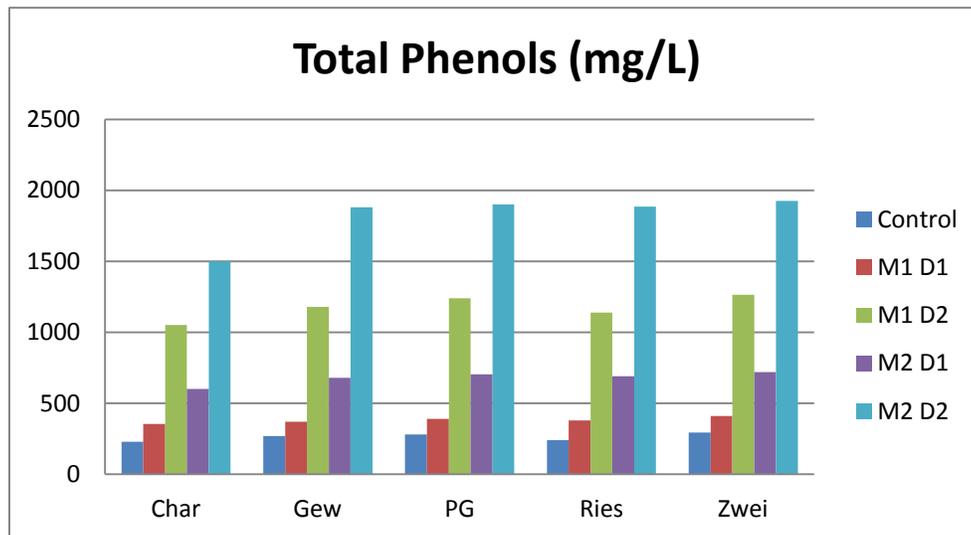
| TA (g/L) | | | | | |
|-----------|-------|------|-------|-------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 10.13 | 8.70 | 10.70 | 11.55 | 9.10 |
| M1 D1 | 10.2 | 9.3 | 11.3 | 11.78 | 10.2 |
| M1 D2 | 10.13 | 9.2 | 10.4 | 12.08 | 10.3 |
| M2 D1 | 10.6 | 10.6 | 10.1 | 13.8 | 10.7 |
| M2 D2 | 10.2 | 10.4 | 10 | 12.9 | 10.1 |



- iv) **Total phenols:** All treatments in all varieties showed higher total phenols as compared to their controls (Table 13). The total phenols content on dose 1 (1.0kg/plant) was recorded lower than dose 2 (2.0kg/plant) for both the mixtures in all the selected varieties.

Table 13: Total phenols analysis of different varieties X treatments

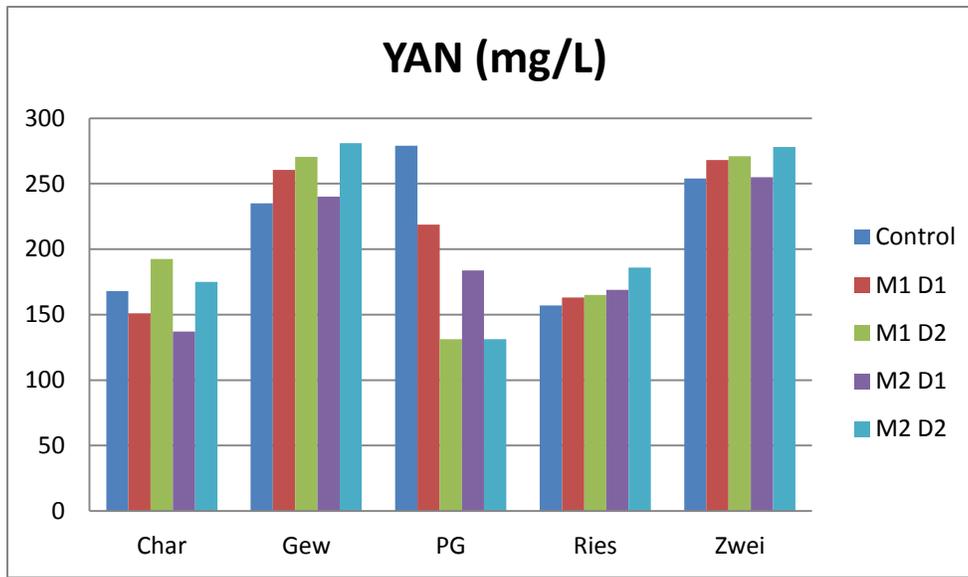
| Total phenols (mg/L) | | | | | |
|----------------------|------|-------|------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 230 | 270 | 280 | 240 | 295 |
| M1 D1 | 355 | 370 | 390 | 380 | 410 |
| M1 D2 | 1052 | 1,180 | 1240 | 1140 | 1265 |
| M2 D1 | 600 | 680 | 705 | 690 | 720 |
| M2 D2 | 1500 | 1,880 | 1900 | 1885 | 1925 |



- v) **Yeast assimilable nitrogen concentration (YANC or YAN):** YAN values for mixture treated plants were higher than controls for Gewürztraminer, Zweigelt and Riesling (Table 14). In case of Chardonnay only treatment M1D2 was higher than control while for Pinot Gris, Control was highest.

Table 14: YAN analysis of different varieties X treatments

| YAN (mg/L) | | | | | |
|------------|-------|--------|--------|------|------|
| Treatment | Char | Gew | PG | Ries | Zwei |
| Control | 168 | 235 | 279 | 157 | 254 |
| M1 D1 | 151 | 260.61 | 218.7 | 163 | 268 |
| M1 D2 | 192.6 | 270.5 | 131.25 | 165 | 271 |
| M2 D1 | 137 | 240.14 | 183.75 | 169 | 255 |
| M2 D2 | 175 | 280.97 | 131.25 | 186 | 278 |



Discussion and recommendations for further study

The present study demonstrates the recycling of viticulture and winery waste & by products (organic wastes) successfully into the organic vineyards. A laboratory study (Nogales *et al.*, 2005) also emphasised great potential in various winery wastes (spent grape marc, vinasse biosolids, lees cakes, and vine shoots) as raw substrates for vermi-composting. Vermi-composting improves the agronomic value of the winery wastes by reducing the C: N ratio, conductivity and phytotoxicity, while increasing the humic materials, nutrient contents and pH. Organic wastes, if applied individually, may show nutrient toxicity in soils or foliar deficiencies in plants. We noticed that application of such decomposed organic wastes mixed with other organic products like cow manure and saw dust was much safer to plants and environment. Ozdemir (2008) also obtained similar results using different organic manures (farm yard manure, pruning residues, straw mulch and green manure). The macro and micro nutrient levels in leaf samples were higher than controls. Similar findings were also reported by Pinnamonti *et al.* (1999) for variety Merlot. The compost derived from sewage sludge and bark with low heavy metals was beneficial than compost obtained from municipal solid waste origin accumulating heavy metals in soils, leaves and grapes. In our experiment, there was a visual improvement in plant vigor and crop yield characteristics though with comparable quantitative benefits except varietal differences. The possibilities could be slow release of nutrients from the mixtures especially nitrogen, insufficient uptake hampered by varied soils types, moisture contents in soils and ionic imbalance across the root cell membranes, in the vineyards. Research has shown that much of the nitrogen in compost is initially bound in an organic form and is therefore not readily available to plants right way on application. However, it is constantly and steadily being mineralized into an available form with the passage of time. Nendel and Reuter (2007a) reported, high phenol contents in grape pomace, which contribute slow but steady input of nitrogen in the vineyards. However, in an another vineyard study (Nendel and Reuter, 2007b) on compost application and monitoring of N dynamics in soils, the strong accumulation of mineral nitrogen was found on top soils and showed a translocation downwards throughout the year.

The higher pH values of the juice with both mixtures on dose 2 (2.0kg) could be due to higher levels of Potassium. Though, Potassium (K) is a macro-nutrient important for regulating water movement within the grape vine, and K deficiency in soil can result in reduced vine growth, premature leaf drop, and yield loss. However, oversupply of K may lead to lower tissue calcium and magnesium and higher grape juice pH. Similar results were reported by Chan and Fahey, 2011. Morlat and Symoneaux (2008) reported increased use of organic amendments specially cattle manures increase the pH, TA and YAN of grape juice which corroborate with our findings. Similar data on higher total phenols on berry maturity were also reported by Saber *et al.* (2010). Nielson *et al.* (2010) suggested the timing and form of nitrogen greatly influence YAN in grape juice.

Recommendations for further study

- Continued evaluation of experimental treatments in the second year for plant vigor, crop quantity and juice quality
- Petiole or leaf analysis for assessment of macro and micro-nutrients during developmental stages in the next year
- Soil analysis in the beginning of next season of treatment plots for nutrient assessment

Acknowledgements

Kalala Organic Vineyards Ltd. is thankful to Organic Sector Development Program, COABC, for financial assistance for the present study.

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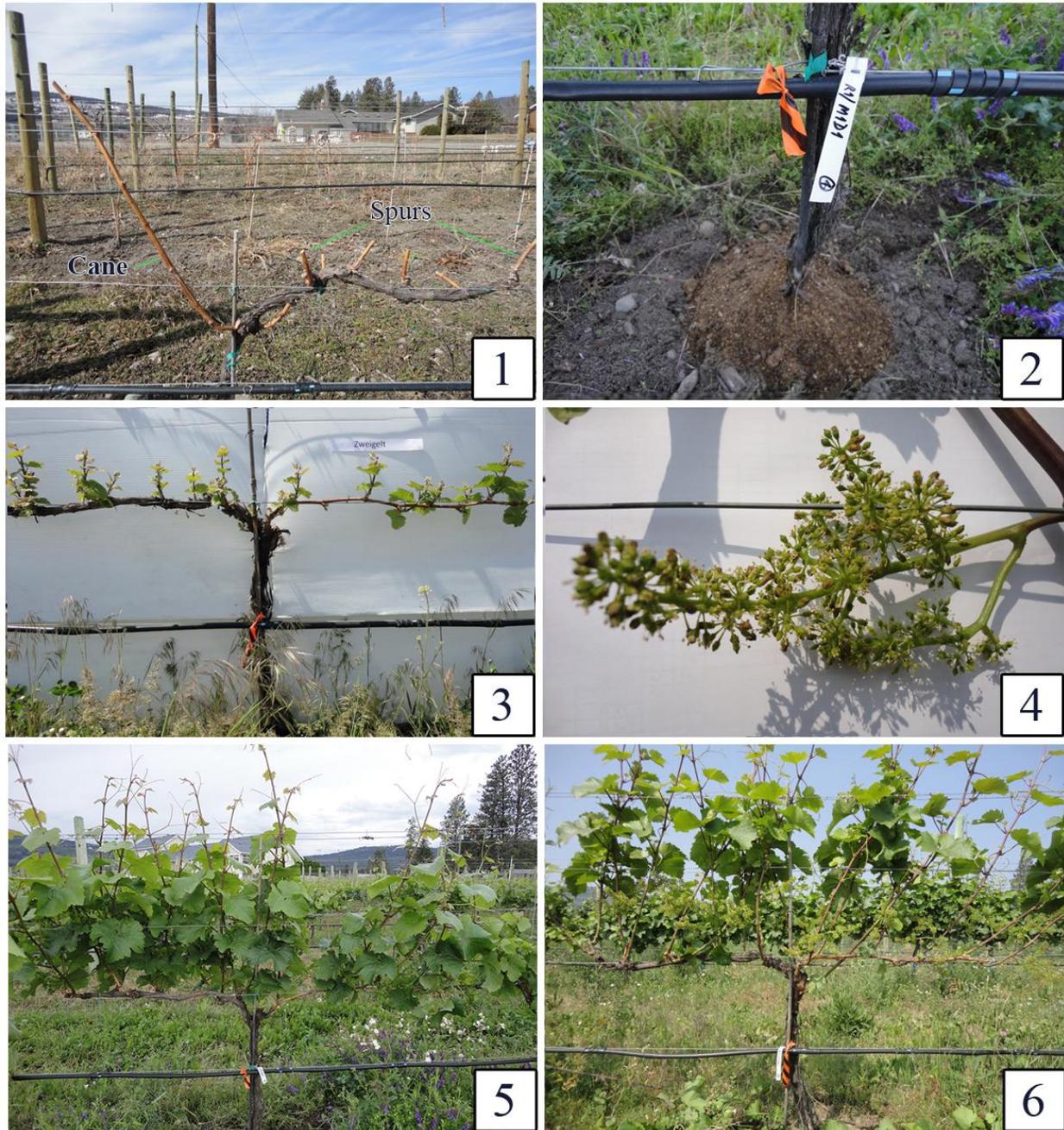


Figure 1:

1. Pruning (cane and spur) in Gewurztraminer
2. Marking of plants with treatment codes and application of organic mixture
3. Bud break frequency in Zweigelt
4. Flowering and berry development in Zweigelt
5. Cane formation in Zweigelt
6. Zweigelt plant after leaf thinning

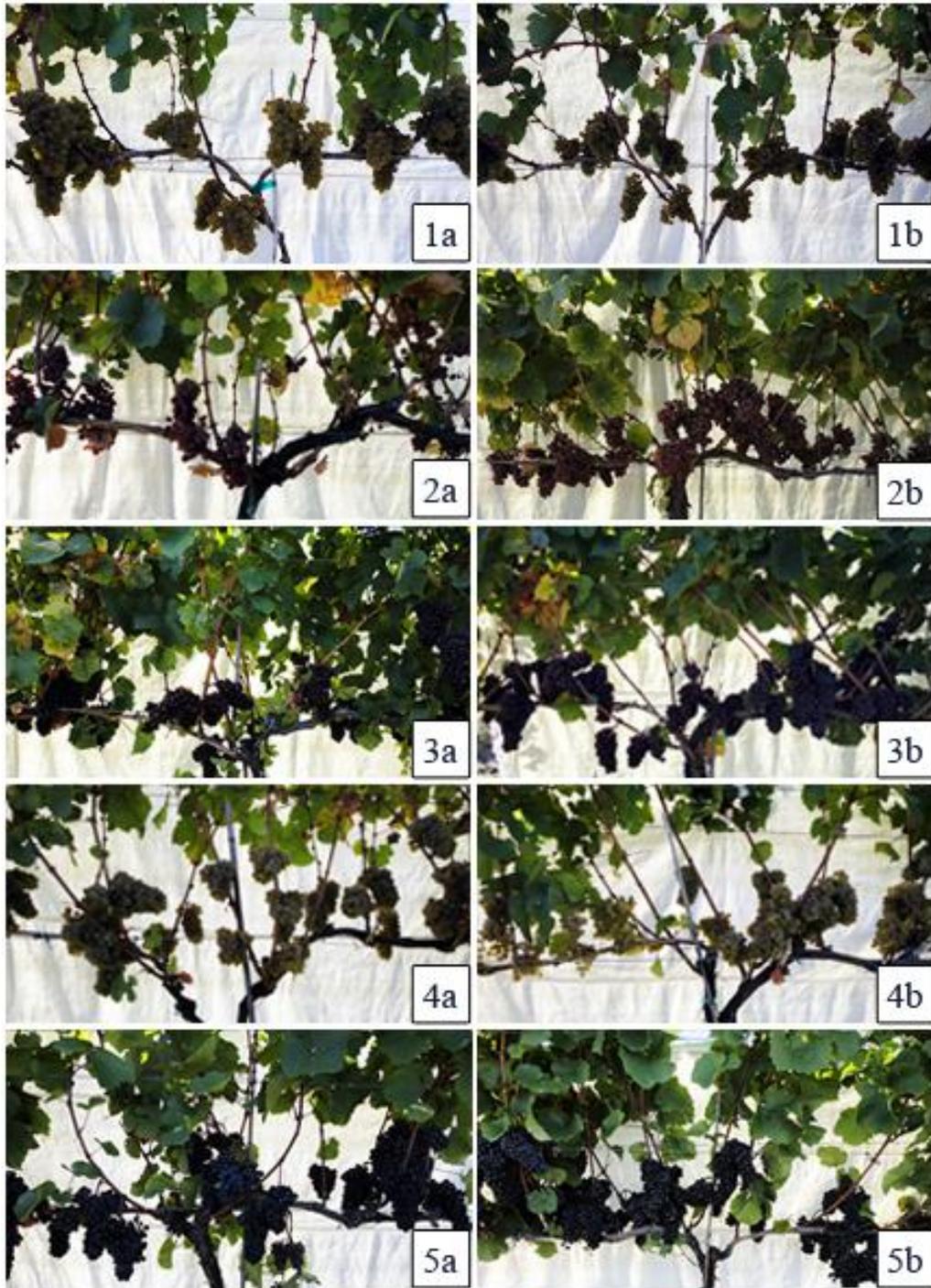


Figure 2:

- 1a Chardonnay - Control; 1b - Chardonnay Treatment M1D1
- 2a Gewurztraminer - Control; 2b - Gewurztraminer Treatment M1D1
- 3a Pinot Gris - Control; 3b - Pinot Gris Treatment M1D1
- 4a Riesling - Control; 4b - Riesling Treatment M1D1
- 5a Zweigelt - Control; 5b - Zweigelt Treatment M1D1

**Please see attached
Annexure 1
(Organic mixtures analysis report)**

Report Transmission Cover Page

| | | |
|--|------------------------|-----------------------------|
| Bill To: Kalala Organic Vineyards Ltd. | Project: | Lot ID: 874696 |
| Report To: Kalala Organic Vineyards Ltd. | ID: | Control Number: |
| 3361 Glencoe Road | Name: Organic Mixtures | Date Received: Jun 7, 2012 |
| West Kelowna, BC, Canada | Location: | Date Reported: Jun 19, 2012 |
| V4T 1M1 | LSD: | Report Number: 1742662 |
| Attn: Dave Ashish | P.O.: | |
| Sampled By: | Acct code: | |
| Company: | | |

| Contact & Affiliation | Address | Delivery Commitments |
|-------------------------------|--|---|
| Dave Ashish | 3361 Glencoe Road | On [Report Approval] send |
| Kalala Organic Vineyards Ltd. | West Kelowna, British Columbia V4T 1M1 | (Test Report) by Email - Single Report |
| | Phone: (250) 768-9700 | On [Lot Approval and Final Test Report Approval] send |
| | Fax: null | (Invoice) by Email - Single Report |
| | Email: ashishdave21@hotmail.com | |

Notes To Clients:

- Compost analysis was performed by a subcontract laboratory. See attached 3 page report 1211356.

Methodology and Notes

| | | | | | |
|-------------|-------------------------------|------------|------------------|-----------------|---------------|
| Bill To: | Kalala Organic Vineyards Ltd. | Project: | | Lot ID: | 874696 |
| Report To: | Kalala Organic Vineyards Ltd. | ID: | | Control Number: | |
| | 3361 Glencoe Road | Name: | Organic Mixtures | Date Received: | Jun 7, 2012 |
| | West Kelowna, BC, Canada | Location: | | Date Reported: | Jun 19, 2012 |
| | V4T 1M1 | LSD: | | Report Number: | 1742662 |
| Attn: | Dave Ashish | P.O.: | | | |
| Sampled By: | | Acct code: | | | |
| Company: | | | | | |

Comments:

- Compost analysis was performed by a subcontract laboratory. See attached 3 page report 1211356.

Please direct any inquiries regarding this report to our Client Services group.

Results relate only to samples as submitted.

The test report shall not be reproduced except in full, without the written approval of the laboratory.

Client: Exova Canada Inc. (Surrey)
#104, 19575 - 55A Avenue
Surrey, BC
V3S 8P8
Attention: Exova Surrey
PO#: 512839
Invoice to: Exova Canada Inc. (Surrey)

Report Number: 1211356
Date Submitted: 2012-06-08
Date Reported: 2012-06-18
Project: 874696
COC #: 753592

Dear Exova Surrey:

Please find attached the analytical results for your samples. If you have any questions regarding this report, please do not hesitate to call (613-727-5692).

Report Comments:

APPROVAL: _____

Lorna Wilson
Inorganic Laboratory Supervisor

Exova (Ottawa) is certified and accredited for specific parameters by:
CALA, Canadian Association for Laboratory Accreditation (to ISO 17025), OMAF, Ontario Ministry of Agriculture, Food and Rural Affairs(for farm soils), Licensed by Ontario MOE for specific tests in drinking water.

Please note: Field data, where presented on the report, has been provided by the client and is presented for informational purposes only.

Client: Exova Canada Inc. (Surrey)
 #104, 19575 - 55A Avenue
 Surrey, BC
 V3S 8P8
 Attention: Exova Surrey
 PO#: 512839
 Invoice to: Exova Canada Inc. (Surrey)

Report Number: 1211356
 Date Submitted: 2012-06-08
 Date Reported: 2012-06-18
 Project: 874696
 COC #: 753592

| Group | Analyte | MRL | Units | Guideline | Lab I.D. Sample Matrix Sample Type Sampling Date Sample I.D. | 962658 Compost 2012-06-07 874696-1 M-1 (Organic Matter @550C) | 962659 Compost 2012-06-07 874696-2 M-2 (Organic Matter @550C) |
|-------------------|-------------------------|------|-------|-----------|--|---|---|
| Agri. - Soil | Electrical Conductivity | 0.05 | mS/cm | | | 3.87 | 2.04 |
| | N-NH3 | 1 | ppm | | | 30 | 3 |
| | N-NO3 | 25 | ppm | | | | <25 |
| | | 50 | ppm | | | 150 | |
| | Organic Matter (@550C) | 0.1 | % | | | 28.8 | 29.2 |
| | pH | 2.0 | | | | 8.7 | 8.4 |
| General Chemistry | Moisture | 0.1 | % | | | 37.2 | 29.4 |
| Mercury | Hg | 0.1 | ug/g | | | <0.1 | <0.1 |
| Metals | As | 1 | ug/g | | | 3 | 2 |
| | Ca | 100 | ug/g | | | 13000 | 13000 |
| | Cd | 0.5 | ug/g | | | <0.5 | <0.5 |
| | Co | 1 | ug/g | | | 11 | 11 |
| | Cr | 1 | ug/g | | | 33 | 39 |
| | Cu | 1 | ug/g | | | 26 | 24 |
| | K | 100 | ug/g | | | 14200 | 10700 |
| | Mg | 100 | ug/g | | | 7200 | 7600 |
| | Mo | 1 | ug/g | | | 1 | 1 |
| | Na | 100 | ug/g | | | 700 | 600 |
| | Ni | 1 | ug/g | | | 24 | 27 |
| | Pb | 1 | ug/g | | | 9 | 12 |
| | Se | 1 | ug/g | | | <1 | <1 |
| Zn | 1 | ug/g | | | 72 | 75 | |
| Nutrients | Total Kjeldahl Nitrogen | 0.05 | % | | | 1.14 | 0.44 |
| Others | TOC | 0.01 | % | | | 17.0 | 15.7 |
| | Total P | 0.01 | % | | | 0.21 | 0.15 |
| Ratios | C/N Ratio | | | | | 14.9 | 35.7 |

Guideline = * = **Guideline Exceedence**
 Results relate only to the parameters tested on the samples submitted.
 Methods references and/or additional QA/QC information available on request.

MRL = Method Reporting Limit, AO = Aesthetic Objective, OG = Operational Guideline, MAC = Maximum Acceptable Concentration, IMAC = Interim Maximum Acceptable Concentration, STD = Standard, PWQO = Provincial Water Quality Guideline, IPWQO = Interim Provincial Water Quality Objective.

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Report Number: 1211356
Date Submitted: 2012-06-08
Date Reported: 2012-06-18
Project: 874696
COC #: 753592

Sample Comment Summary

Sample ID: 962658 874696-1 M-1 (Organic Mixture-1) N-NO3 MRL elevated due to matrix interference. TKN was analysed as received and reported on dried sample basis for both samples.

Guideline = *** = Guideline Exceedence**
Results relate only to the parameters tested on the samples submitted.
Methods references and/or additional QA/QC information available on request.

MRL = Method Reporting Limit, AO = Aesthetic Objective, OG = Operational Guideline, MAC = Maximum Acceptable Concentration, IMAC = Interim Maximum Acceptable Concentration, STD = Standard, PWQO = Provincial Water Quality Guideline, IPWQO = Interim Provincial Water Quality Objective.