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Assessment for diseases index in downy mildew (*Plasmopara viticola*) in field-grown grapevine with some Natural Compounds in Albania

Suela Spahiu[1]; Hekuran Vrapi[2*].Thanas Ruci[2].
[1*] *(PhD Candidate), Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, Kodër Kamëz, Tirana, Albania*
[2]. *Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, Kodër Kamëz, Tirana, Albania,*
**Correspondence author: Hekuran Vrapi*

Abstract: *The investigation was carried out during 2011.2012 and 2017 seasons in Marikaj in Tirane Capital, Tirane – Durrës desert road under natural infection conditions. The efficacies of Solfato di rame 20%, Algae extract, Clay with Al-sulfate, Algae extract with potassium \ Phosphomat and alternate, against downy mildew were studied on grapevine cv. Merlot. All the tested compounds provided protection at different level against downy mildew* **[***Plasmopara viticola (Berk. et Curtis ex. de Bary) Berl.] on both leaves and bunch as compared with contoll which record the highest effect. Solfato di rame 20%, Alternate, Clay with Al-sulfate and Algae extract with potassium \ Phosphomat were more effective on leaves and bunch as compared with used in a single treatment. From the data we see that the lowest index on the leaf and thus coat the Imc in% of the* downy mildew *(Plasmopara viticola (Berc and Curt) have the variants where we have used Biological Alternative products and Cuprozin fl respectively Imc = 9.5% and 11.5% in leaf as well as 4.94% and 5.58% in coats compared to the control variant that is 66% in leaves and 57.5% in coats. Satisfactory results have yielded Bioproducts such as Muco-Sin, Frutogard and Algin Biovital, respectively Imc = 15%, 16.6% and 17% in leaf as well as 9.91%, 13.08% and 11.83% in Woolly compared to the control Which in Imc's leaf reaches 66% and wears 57.5%.*

Key words: *Grapevine, Plasmopara viticola, Solfato di rame 20%, Algae extract, Clay with Al-sulfate, Algae extract with potassium \ Phosphomat*

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I. Introduction

Grapevine (Vitis vinifera), a major fruit crop worldwide, is affected by many diseases, such as downy mildew (Plasmopara viticola), powdery mildew (Erysiphe necator) and grey mould (Botrytis cinerea). Against these severe diseases, modern sustainable viticulture aims to limit chemical treatments by using alternative strategies. One of them is to trigger grapevine resistance by eliciting its innate immunity.

The downy mildew of grapevine is one of the most destructive diseases in viticulture resulting in severe epidemics and enormous economic costs. The causal agent is *P. viticola* (Berk. and Curt.) Berl. and de Toni, an obligate biotrophic oomycete of the Peronosporaceae family, which was introduced to Europe from North America in the 1870s [1].

In organic farming, the control of *P. viticola* is based on the use of copper, together with all preventive management measures necessary to minimize the development of disease. Copper is still a very important tool to manage the diseases in conventional agriculture and is actually indispensable in organic farming (24, 21). With Commission Directive 2009/37/EC, copper compounds have been included in Annex I to Directive 91/414/EEC (concerning the placing of plant protection products on the market). “Therefore, it is necessary that

An organic vineyard is a complex living system where the grower actively tries to encourage the self regulation of the ecosystem and the health of this organism. In organic viticulture one of the primary goals is to grow healthy and disease tolerant or resistant plants.

The European and Mediterranean organization for plant protection established the standard for the best practice regarding grapevine protection that includes the listed procedures and products, as well as the lists of the most significant diseases and grapevine pests (OEPP/EPPO, 2000). The lists of the allowed pesticides for organic production in the European countries are integrated in the following directives-Regulation (EEC) No. 2092/91 (1991) - ANNEX II, Regulation (EC) No. 834/2007, Article 16(3) (c) and Regulation (EC) No. 834/2007.

There are few data relating to organic production of grapevine in locally published literature and even fewer data treating the protection problems. The objective of this paper is to describe the products for grapevine protection that are used in different countries worldwide in organic production of grapevine.

Downy mildew is one of the most harmful grapevine diseases in every European wine growing zone, the epidemics of which can cause tangible damage both to the leaves and to the bunches. The pathogen can infect all of the vegetative organs of the vine such as leaf, tip, flower, cluster, stalk and young fruit, and can cause numerous infections during the season.

Furthermore, the use of pre- and post harvest chemical treatments is increasingly limited due to consumer concerns. So, this study aims to evaluate The efficacies of Solfato di rame 20%, Alternate, Clay with Al-sulfate and Algae extract with potassium \ Phosphomat were more effective on leaves and bunch as compared with used in a single treatment.

II. Materials And Methods

Plant materials:

The investigation was carried out during 2011,2012 and 2017 seasons in Marikaj in Tirane Capital, Tirane – Durrës desert road under natural infection conditions. The vines were planted in a light calcareous sandy soil and placed at 1.5 m (between vines in the row) x 2.75 m (between rows). The vines were trained to the modified cane training and supported by telephony trellis system. All vines received the same agricultural practices already applied in the vineyard.

Vineyard trials:

The vineyard experiments were arranged in a randomized complete block design with four replications for each variant, four vines for each replicate. The treatment programmers were applied at 15 day intervals during the growing season starting at 10-15 cm shoot length. Tested compounds (Table 1) were sprayed with a Knapsack sprayer. In order to provide a homogeneous spread on the leaf and bunch surface, surfactant triton was added at the dose of 50 ml / 100 L water. Control vines were sprayed with water.

Table 1. Commercial name, active ingredient, dosage and number of applications of the products tested against grape downy mildew

Biofungicides against downy mildew [*Plasmopara viticola* (Berk. et Curtis ex. de Bary) Berl.]				
No	**Chimical trade name**	**Active ingredient**	**Formulation**	**Usage dosage/100 L**
1	Cuprozin fl	Copper- hydroxide	wp	350gr/100l
2	Algin Biovital	Algae extract	liquid	250ml/100l
3	Muco-Sin	Clay with Al-sulfate	wp	250gr/100l
4	Frutogard	Algae extract with potassium Phosphomat	liquid	250ml/100l

Disease assessment:

The disease severity on leaves and bunches were evaluated 12 days after the last application. Bunch infection was evaluated based on a scale of 0 – 4 (Delen et al. 1987) on 72 bunches for each replication, where 0 = no infection on the bunch (no), 1 = 25% infection (n1), 2 = 50% infection (n2), 3 = 75% infection (n3), and 4 = more than 75% infection (n4).

Leaf infection was evaluated based on a scale of 0 - 3 (Delen et al. 1987) on 150 leaves for each replication, where 0 = no colony on the leaf (n0), 1 = 1 - 2 colonies per leaf (n1), 2 = 3 - 10 colonies per leaf (n2), and 3 = more than 10 colonies per leaf (n3). The experiments were established as randomized complete block design with 4 replications, and 8 plants were used for each plot. Main plots include spraying treatments, while the sub-plots include pruning. The distance between plots was 3 m.

Statistical Date Analysis

Disease severity (%) was evaluated using Townsend-Heuberger formula. The effectiveness of spray programs was evaluated using the Abbott formula, and the resulting data were assessed using Minitab® Statistical Software Release 14. The mean values were compared by Duncan’s multiple range test at P < 0.05.
The efficiency of chemical and fungicidal treatments was calculated by the following formula: Efficacy of treatment = ((control-treatment)/control) 100, Mousa, *et al*. 2006.

III. Results

IV. 4.1 RESULTS ON THE INDEX OF DISEASE FOR POWDERY MILDEW (PLASMOPARA VITICOLA (BERC E CURT) THREE YEARS OF THE STUDY :(YEAR 2011-2012 AND 2017)

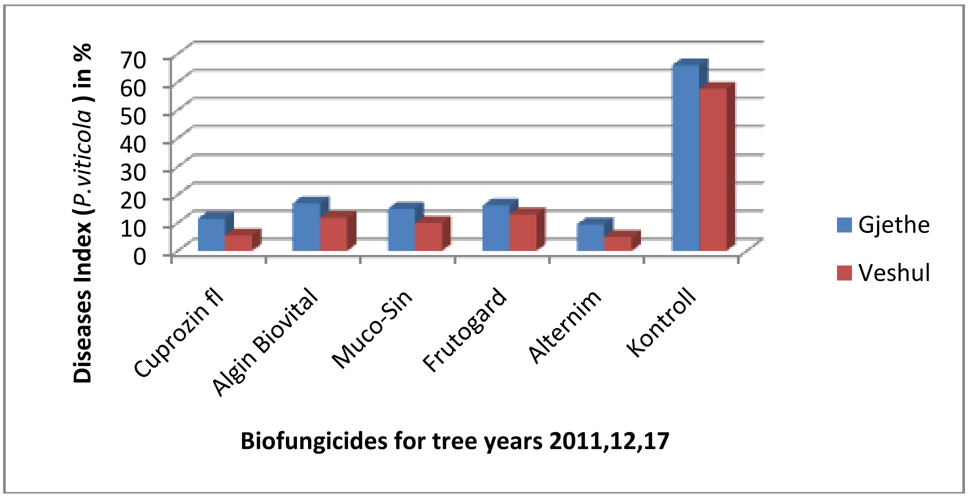
Initial data on the results achieved on the disease index (Imc in%) of the downy mildew (Plasmopara viticola (Berc and Curt) on leaves and dress for the six variants in the biological test for each study year are given in **Tables 8.4, 8.5** and 8.6 (located in the Annex that can’t be seen in this article) as well as in the Summary Table for the three years in the study **Table 4.1**, Graph 4.1 and Figure 4.1.

Table 4.1. On the evaluation of the disease index (Imc in%) in leaf and wax against downy mildew (**Plasmopara viticola** (Berc e Curt) for the three years in the 2011.12 and 2017 study at the Bio farm in the village of Marikaj in Tirana.

Assessment		x			□ Imein %	Average Disease severity	Dunnett's Test*
		2011	**2012**	2017			
Assessment in the leaves	Cuprozin fl	16	11	7.5	34.5	11.5	C
	Algin Biovital	20.5	18	12.5	51	17	B
	Muco-Sin	18	17	10	45	15	B
	Frutogard	18	18	12.5	48.5	16.16	B
	Alternim	10.25	14	4.25	28.5	9.5	C
	Control	63.75	75	59.25	198	66	A
	MEANS	146.5	153	106	405.5		
	AVERAGE	24.416	**25.5**	17.66		22.52	
	DMV =3.35886 per @=0.05						
Assessment in the bunches	Cuprozin fl	5	7	4.75	16.75	5.58	C
	Algin Biovital	11.5	14	10	35.5	11.83	B
	Muco-Sin	9	11	9.75	29.75	9.91	BC
	Frutogard	13.5	14	11.75	39.25	13.08	B
	Alternim	3.5	8.08	3.25	14.83	4.94	C
	Control	56.25	60	56.25	172.5	57.5	A
	MEANS	98.75	114.08	95.75	308.58		
	AVERAGE	16.45	**19.01**	15.95		17.14	
	DMV =3.35886 per @=0.05						

Note: A lower detection level for DMV = 3.35886 for @ = 0.05 and B = highest detection level for DMV = 3.35886 for @ = 0.05 according to the Dunnett test

From the data in **Table 4.1** we see that the lowest index on the leaf and thus coat the Imc in% of the downy mildew (*Plasmopara viticola* (Berc and Curt) have the variants where we have used Biological Alternative products and Cuprozin fl respectively Imc = 9.5% and 11.5% in leaf as well as 4.94% and 5.58% in coats compared to the control variant that is 66% in leaves and 57.5% in coats.
Satisfactory results have yielded Bioproducts such as Muco-Sin, Frutogard and Algin Biovital, respectively Imc = 15%, 16.6% and 17% in leaf as well as 9.91%, 13.08% and 11.83% in Woolly compared to the control Which in Imc's leaf reaches 66% and wears 57.5%.



Graph 4.1. On the Evaluation of the Infectious Disease Index (Imc in%) on leaves and wounds against the downy mildew (*Plasmopara viticola* (Berc and Curt) for the three years in the 2011.12 and 2017 study at the Bio farm in the village of Marikaj in Tirana.

From the data in **Graph 4.1** and adjusted in **Figure 4.1** we see that the lowest columns in comparison to the control therefore the lowest index as in the leaf and thus wrap the Imc in% to the downy mildew (Plasmopara viticola (Berc e Curt) have variants where we used Biological Alternative products and Cuprozin fl

= Imc = 9.5% and 11.5% in leaves and 4.94% and 5.58% in coat compared to control variant which is 66% in leaves and 57.5% in bunch.

Satisfactory results have yielded Bioproducts such as Muco-Sin, Frutogard and Algin Biovital, respectively Imc = 15%, 16.6% and 17% in leaf as well as 9.91%, 13.08% and 11.83% in Wearing compared to the control Which in Imc's leaf reaches 66% and wears 57.5%.

The variance analysis (ANOVA factor) for the study described in the tables 8.7, 8.8 and 8.9, set out in the Annex, shows the variability between the test variants (treatments) for both levels of veracity (0.01 = <p <0.05). The fact that duplicates are not verified for both levels of authenticity (0.01 = <p <0 .05) allows us to proceed further with the judgment. The variance analysis (ANOVA a factoric) (See Table 4.2) shows that for both leaf and coat treatments we have statistically confirmed variance for both levels of P = 0.01 and P = 0.05 This is proven by the factual F values for leaf treatments = 150.6231 ** which results to be greater than the value of the theoretical table F under Fisher where 150.6231 **> 3.325 for the level of veracity < 0.05 and greater than the value of the theoretical table F under Fisher where 150.6231 **> 5.63632 for the level of veracity <0.01 This is proven by the factual F values for coagulation treatments = 1509.079 ** which results to be greater than the value of the theoretical table F under Fisher where 1509.079 **> 3.325 for the level of veracity < 0.05 and greater than the value of the theoretical table F under Fisher where 1509.079 **> 5.63632 for the level of veracity <0.01 The fact that the recurrences are the years of study emerged saturated in the ANOVA factorial analysis shows that in our experiment the year factor is important because the vortex of the vine is conditioned by the atmospheric agents of the humidity temperature and the stage of the development of the vegetation. This is proved by the values of the "F" Factors for Foliar Treatment = 11.75325 ** which results to be greater than the value of the theoretical table F under Fisher where 11.75325 ****> 4.1028 per level of Truth <0.05 and greater than the value of the theoretical table "F" according to Fisher where 11.75325 **> 7.55943 for the level of veracity <0.01. This is proven by the factual values of "F" for coatings = 20.17959 ** which results to be greater than the value of the theoretical table F under Fisher where 20.17959 **> 4.1028 for the level of veracity < 0.05 and greater than the value of the theoretical table "F" according to Fisher where 20.17959 **> 7.55943 for the level of veracity <0.01.

Table 4.2. Variances Analysis (ANOVA). On the Evaluation of the Infectious Disease Index (Imc in%) on leaves and wounds against the downy mildew (*Plasmopara viticola* (Berc and Curt) for the three years in the 2011.12 and 2017 study at the Bio farm in the village of Marikaj in Tirana.

Assessment	Variation Source	Df*	Square Sum	Mean Square	Statistics of the test		
					F. actual	< F theory >	
						95%	99%
Leaf	Biofungicides	5	6926.569	1385.314	150.6231**	3.325	5.63632
	Years	2	216.1944	108.0972	11.75325**	4.1028	7.55943
	Error	10	91.97222	9.197222			
	Total Variation	17	7234.736				
Bunch	Biofungicides	5	6024.116	1204.823	1509.079**	3.325	5.63632
	Years	2	32.2221	16.11105	20.17959**	4.1028	7.55943
	Error	10	7.983833	0.798383			
	Total Variation	17	6064.322				

Note **: Verified for propensity level 1% (p <0.01); *: Verified for propensity level 5% (0.01 = <p <0.05); NS: Without proof of profitability (p> = 0 .05)

We also carry out the Analysis of variance (two factor) MANOVA for our experiment where we have taken as factor A = three-level years and Factor B = 6-level variants for four repetitions using the Statistical Assistant (The Assistant Software Version 7.7, and its use in the analysis of experimental data, F. J. Agric Res. Vol. 11 (39), pp. 3733-3740, September 29. DOI: 10.5897 / AJAR2016.11522 from Silva FAS, Azevedo CAV (2016). Is reflected in the table below (see table 4.6)

Table 4.3 Factorial Analysis (two factor) MANOVA. On the Evaluation of the Infectious Disease Index (Imc in%) on leaves and wounds against the downy mildew (*Plasmopara viticola* (Berc and Curt) for the three years in the 2011.12 and 2017 study at the Bio farm in the village of Marikaj in Tirana.

Assessment	Variation Source	Df*	Square Sum	Mean Square	F factual	Probability
Leaf	Years (A)	2			90.8760 **	<.0001
	Treatment B	5			1204.7880 **	<.0001
	Int AxB	10	399.88889	39.98889	8.6589 **	<.0001

	Treatments	17	29129.944	1713.52614	370.1216 **	<.0001
	Error	54	250	3.99074	-	-
	Total Variation	71	29379.944	-	-	-
Bunch	Years (A)	2	125.1025	62.55125	11.7203 **	<.0001
	Treatment B	5	24193.14	4838.62792	906.6210 **	<.0001
	Int AxB	10	33.42917	3.34292	3.6264 *	0.7847
	Treatments	17	24351.671	1432.45125	268.4005 **	<.0001
	Error	54	288.1975	5.33699		-
	Total Variation	71	24639.869			-

Note *Df = Degree of freedom ** Significative at a level of 1% of probability (p < .01) * Significative at a level of 5% of probability (.01 =< p < .05) ns Non-significative (p >= .05)

From the Analysis of variance (two factor) MANOVA (See Table 4.3) shows that Factor A (three-year-olds) Factor B treatments (at 6 levels) as well as interaction A x B as in leaves and coats have statistically confirmed variability For both levels of P = 0.01 and P = 0.05 This is proved by the factual F values for Factor Variables in Fennel Assessments = 90.8760 ** which results to be greater than the value of the theoretical table F under Fisher where 90.8760 **> 3.325 per level The truth <0.05 then the critical F criterion and the proven with a high level of propiability for P <. 0001. This is proven by the factual F values for the Factor Treatment on Flax Evaluation = 1204.7880 ** which results to be greater than the value of the theoretical table F under Fisher where 1204.7880 ** <3.325 per level The truth <0.05 then the critical F criterion as well as the proven with a high level of propensity for P <0001 This is proven by the values of the "F" factor for the AxB Coefficient factor in the leaf estimates = 8.6589 ** which results to be greater than the value of the theoretical table "F" according to Fisher where 8.6589 ** <3.325 . The level of authenticity <0.05 so critical F as well as the proven with a high level of propiability for P <0001. This is proven by the factual F values for the Factor Variable in Veshul = 11.7203 ** which results to be greater than the value of the theoretical table F under Fisher where 11.7203 **> 3.325 per level The truth <0.05 then the critical F criterion and the proven with a high level of propiability for P <. 0001

This is proven by the factual F values for the Vaginal Wear Treatment factor = 906.6210 ** which results to be greater than the value of the theoretical table F under Fisher where 906.6210 ** <3.325 per level The truth <0.05 then the critical F criterion as well as the proven with a high level of propensity for P <0001. This is proven by the factual F values for the AxB Coefficient factor in Wear = 3.6264 * which results to be greater than the value of the theoretical table F under Fisher where <3.325 for the level of veracity < 0.05 Well F Critics

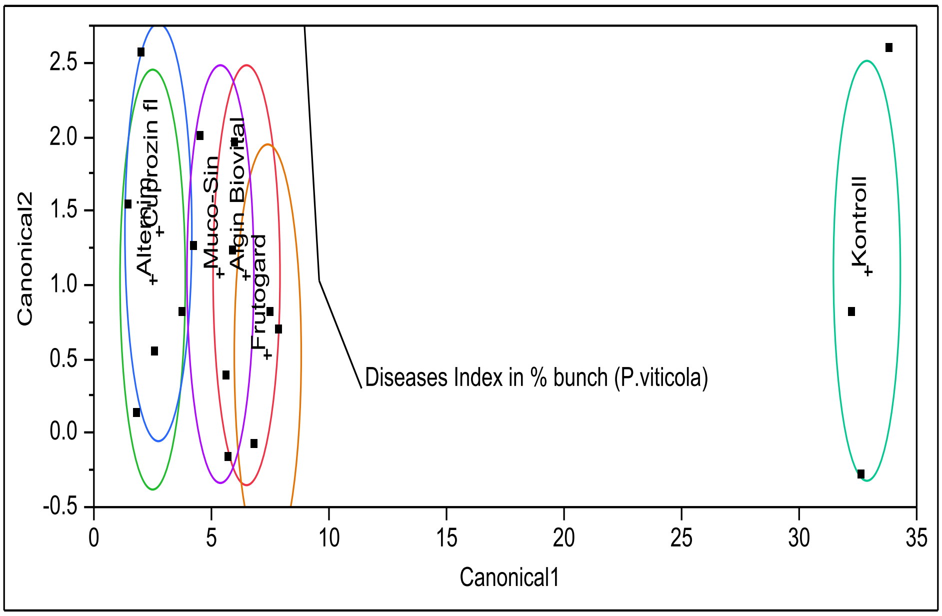


Figure 4.1. Adaptation of both indexes of disease (Imc in%) in leaves and wounds against the downy mildew (*Plasmopara viticola* (Berc e Curt) for the three years in the 2011.12 and 2017 study at the Bio farm in the village of Marikaj in Tirana.

Illustrating these changes of the biofungicides used (treatments) for the Influenza Index (Imc in%) in leaf and coat against the downy mildew (Plasmopara viticola (Berc e Curt) for the three years of study at the Bio farm in the village of Marikaj Tiranë Are given in Figures 4.2 and 4.3 where biofungicides having variability and statistically verified for the propensity level P = 0.05 are blue circles and exactly the Cuprozin fl, Algin Biovital, Muco-Sin, Frutogard and alternatives which are below the mean value of For leaf evaluation in the value of 22.52% and in the ears for the value of 17.4%.

Comparisons of Imc in% Mean Control with the Dunnett Test DMV = 3.35886 for @ = 0.05 shows the presence of the proven variations between the variants in the study where the best variants are the treatments with the Cuprozin fl and alternation variants Which are labeled with the letters C followed by the biofungicides Algin Biovital, Muco-Sin, Frutogard that are marked with the B mark in comparison with the control A which is the lowest level of DMV = 3.35886 for @ = 0.05 for the evaluation on the leaves See Table 4.1 and Figure 4.2)

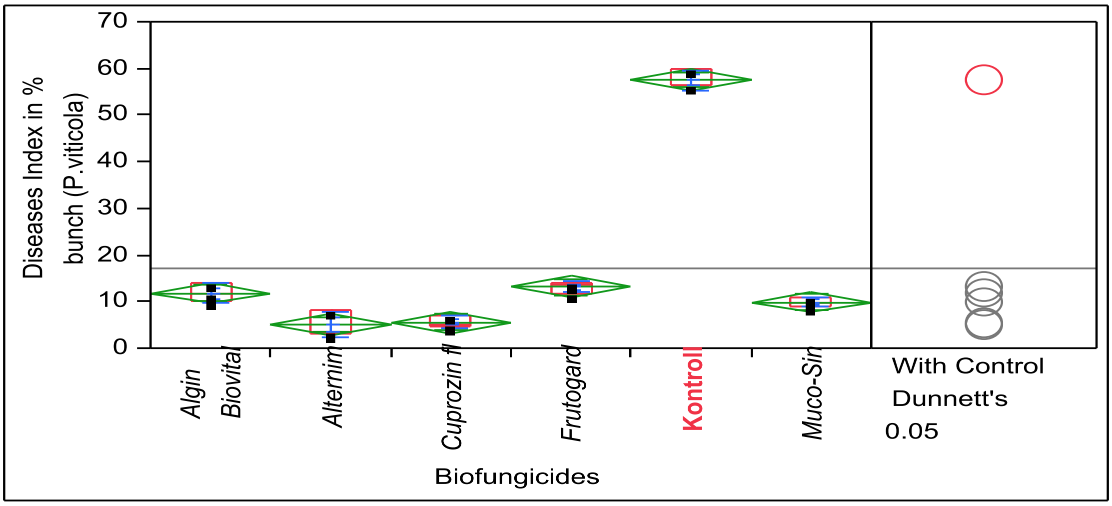


Figure 4.2. Oneway Analysis of Diseases Index in% bunch downy mildew (P. Viticola) By Biofungicides

For VCT estimation Imc Comparisons in% control treatment averages using Dunnett test DMV = 3.35886 for @ = 0.05 indicates the presence of proven variations among the variants in the study where the best variants are treatments With the Cuprozin fl and the Alternative variants that are labeled with the C letters followed by the biogenic algin Biovital, Frutogard, which are labeled with the B-Muco-Sin, with the BC mark in comparison to control A, which is the lowest level of DMV = 3.35886 for @ = 0.05 for leaf estimation (see Table 4.1 and Figure 4.3)

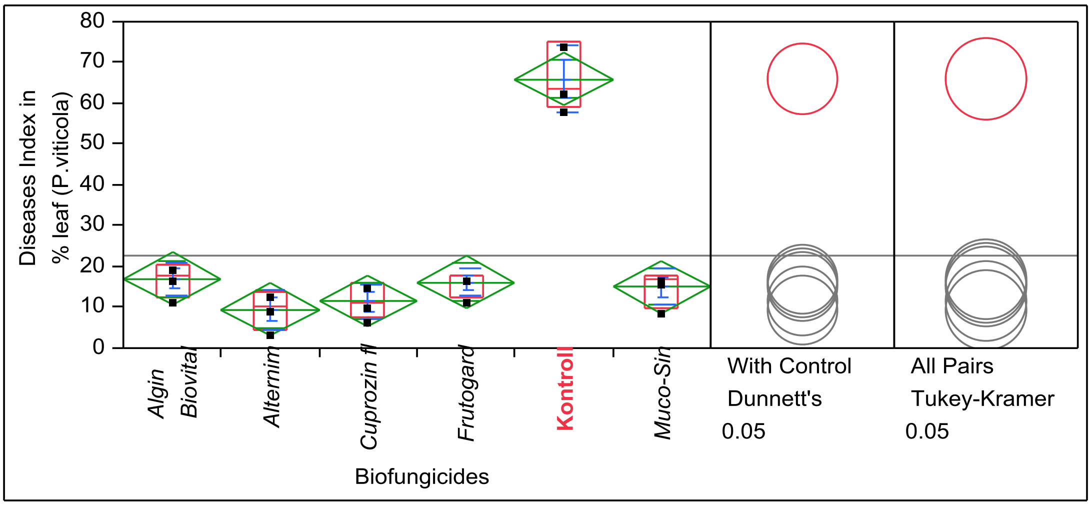


Figure 4.3. Oneway Analysis of Diseases Index in % leaf downy mildew (P.viticola) By Biofungicides

IV. Conclusions

Based on the results presented above in the terms of a Bio farming all tested products have been effective in controlling for downy mildew [Plasmopara viticola (Berk. et Curtis ex. de Bary) Berl.] The best results under our experiment were those with the copper-based formulation Cuprozin fl ,Muco-Sin and the one with alternation (where all the biofungicides in the study were alternated). But seeing the results of natural prodigies like Muco-Sin, Algin Biovital and Frutogard are able to control well the vultures of vines downy mildew [Plasmopara viticola (Berk. et Curtis ex. de Bary) Berl.] From the data we see that the lowest index on the leaf and thus coat the Imc in% of the downy mildew (Plasmopara viticola (Berc and Curt) have the variants where we have used Biological Alternative products and Cuprozin fl respectively Imc = 9.5% and 11.5% in leaf as well as 4.94% and 5.58% in coats compared to the control variant that is 66% in leaves and 57.5% in coats. Satisfactory results have yielded Bioproducts such as Muco-Sin, Frutogard and Algin Biovital, respectively Imc = 15%, 16.6% and 17% in leaf as well as 9.91%, 13.08% and 11.83% in Woolly compared to the control Which in Imc's leaf reaches 66% and wears 57.5%.

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