

Using compost of grape manufacture and farm wastes as growing media in vegetable and ornamental nurseries

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Summary: This work was conducted at private nursery in Kafr El-Sheikh governorate during 2006 and 2007 seasons to investigate the possibility of using grape manufacture waste compost (GMWC) and farm wastes compost (FWC) in ornamental and vegetable nurseries as partially or totally replacement of coconut peat (CP) and vermiculite (V) in the growing medium and also to find out the optimum media of tomato (*Lycopersicon esculentum*, cv. Castle Rock) and Cockscomb (*Celosia plumosa*) as comparing to a mixture of CP and V (1:1 v/v). The authors used a ten mixtures as followed: 1- Control (CP+V at 1:1 v/v), 2-GMWC (100 %), 3- GMWC +CP (1:1 v/v), 4- GMWC + V(1:1 v/v), 5- GMWC +CP + V (1:1:1 v/v/v), 6- FWC (100 %), 7- FWC+ CP (1:1 v/v), 8- FWC+ V (1:1 v/v), 9- FWC+ CP+ V (1:1:1 v/v/v), 10- GMWC+FWC+CP+V (1:1:1:1 v/v/v/v). Data recorded as seedling height, No. of leaves, total pigments, shoot fresh and dry weights, root length and root fresh and dry weights in order to assess the quality of both transplants of tomato and cockscomb. Both seedlings grown in medium contain a mixture of GMWC+CP+V displayed quality traits similar or better as to those of recorded from the control treatment.

Key words: Grape manufacture waste compost, farm wastes compost, growing media, Seedlings

Introduction

Grape manufacture and farm wastes are one of the major environmental problems in Egypt and many country in the world, with environmental pollution and toxics effects on plants and several hydrobrial organisms, mainly because of its phenol and organic matter contents. The direct incorporation of grape more into agriculture land, a common practice, has proved to be a serious problem since the degradation products can inhibit root growth due to presence of phenol, tannins, etc. (Inbar et al., 1991).

An alternative to overcome problems found in the environmental pollution and direct application of plant in composting of grape and farm wastes and use them as a growing media in the nursery.

One of the most important functions of the soil supplying the plants with oxygen, water, or rather, with nutrients dissolved in water. This function requires the proper ratio of three soil phases (physical, chemical and biological) and is possible only with soil where harmful compaction doesn't occur. (Kappel et al., 2003) Considering of features of soil textures the use of peat moss is the most favorable. (Slezák et al., 2005)

Accordingly the primary components of soilless rooting media for vegetable transplant production are peat moss, perlite, and vermiculite (Raviv et al., 1998; Granberry et al. 2001). Peat is a non-renewable resource and diminishing

availability is prompting price increases. Massive use of peat as a substrate has led vegetable-growers to consider its replacement in the medium to long term (Raviv, 1998; Granberry et al., 2001; Sterrett, 2001; Slezák et al., 2005).

Pot ornamentals are mostly grown in soilless growing media that contain the rather expensive peat mixed with an inorganic material. Composted wastes could be considered as an appreciable low priced organic ingredient for pot ornamental growing media and simultaneously disposal of these wastes in a friendly to the environment (Papafotious et al., 2004).

Coco fibers have a good biostability after 120 days (biostability index BI=92.2%), also, coco fibers are increasing used as substrate because they have many characteristics in common with peat (Lemaire, 1997). However, a very high variability in physical and chemical properties, according to their origin, is often found (Prasad, 1997).

Developing peat alternative substrates in nurseries for three different reasons: the resource of peat are limited, the pressure of using waste coming from human or industrial activities increases rapidly and the economic necessity to use locally produced waste products. The biostability of these materials varies and has consequences on the chemical properties of substrates (Lemaire et al., 1998). These new materials must have stable physical and chemical properties during cultivation, and it is necessary to obtain an adequate

balance between biostability and culture period (Guerin et al., 2001).

According to Diaz et al. (2004) indicated that composting of grape marc and vinasse can be considered as an ecological way of recycling those wastes. They showed that a final product (compost) with acceptable properties (maturity and chemical) entails operating at medium operation time (20–35 days).

The objective of this investigation was to evaluate the use of composts made from grape manufacture waste and farm waste as a media in vegetable and ornamental nurseries as an alternative media to coco peat and vermiculite and to determine and limitation to their use, and also obtain the effect of those media on tomato and celosia transplants during the two experiments.

Materials and methods

The study was carried out through two years (2006-2007) in a private nursery in Kafr El-Sheikh governorate to producing tomato and cockscomb seedlings with alternative media, which tested the alternative substrates in real production condition.

Farm waste compost (FWC) was purchased from commercial source, consisting of 50% vegetable crops waste and 50% rice straw. While GMW compost was made from grape manufacture waste of Roomy Red cultivar on Sept. 30th of 2005, than all of other materials mixed and arranged in heaps at 2 m width × 1.5 m height × 5 m long, which were regularly turned and watered for six months to ensure appropriate composting conditions (turned windrow system). The heaps were covered with plastic to protect it from rain water and to ensure high temperature. Heaps were watered in sprinkler system when it need (at field capacity point) and turned monthly to ensure adequate aeration and high decomposition. Maturity of GMWC was showed when the temperature inside the heap decreased and similar to air temperature around the heap, also decreasing the C/N ratio of compost at the end of composting (after 6 months) comparing with the raw materials. C/N ratio of fresh GMW was 31.8 and it was 15.7 at the end of composting period. So, after that compost dried by air and it was ready to use.

The chemical analysis of two composts was measured immediately before its application and also both of the pH and electrical conductivity (EC) of all substrates mixtures were determined by the soil Department, Fac. Agric., Kafr elsheikh Univ.

Methods used for the analysis of chemical and nutrient characteristics were as follows; pH and EC were determined in aqueous compost

extracts which were prepared by shaking the compost in distilled water at a 1: 10 (w/v) ratio for 2h at room temperature. The suspension was separated by centrifugation and the supernatant was filtered through filter paper. pH and EC were determined in compost extract according to Page et al.(1982).

Media samples were oven dried for 2days at 60 °C and ground to a powder with a ball mill prior to combustion. Dry matter was digested by using a mixture of H₂SO₄ and HClO₄ according to (Jacson, 1967); N- content was measured on dry matter using the Kieldahl Method (Bremnen, 1996). Phosphorus was determined colorimetric spectrophotometry and potassium by flame photometry. Total organic matter (OM) was determined according to (Allison, 1965)

Plastic house experiments were conducted with two plants, one is tomato (*Lycopersicon esculentum* Mill, cv. Castle Rock) and the another one is cockscomb (*Celosia plumose*) were grown to evaluate the suitability of the substrates. Experiments used plastic trays 260 compartments (cell size 2.5 × 2.5 × 7 cm in depth) were filled with a ten media (Table 1). The most expensive component was coconut peat, but it was introduced in this study as a control medium because of its requesting in the nurseries, due to its similarities with peat moss and increasing use as plant growing media in the world. Treatments were arranged in three randomly replicates, each replicate was one tray per each crop (260 cells) and actually occupied 198 cells per tray because the edge cells were not used for data. Two experiments were set up then nursery owing dates were: March 15th and April 1st during 2007. Trays were sown manually, one seed per cell and covered with vermiculite. After sowing trays put in plastic house with temperature ranging from 20 to 30 °C . Nursery trays were watered manually every three days using a hose with sprinkler nozzle, and all media took the same water amount, to maintain the substrate at the field capacity. During the seedlings growth, it were fertilized one time in each experiment after the over emergence by a soluble fertilizer providing 360 N– 360 P₂O₅– 360 K₂O (mg/L). Fertilizer solution was applied with sprinkler system.

Emergence (defined as a seedling with cotyledons visible above the media surface) started after 4 and 5 days from sowing in both of tomato and cockscomb, respectively in the two experiments. Emergence percentages were calculated till

Table 1 Composition of the tested substrates and relative cost

Substrates	Composition	Relative cost of 100 trays(\$)
CP+V (1:1)	Coco peat + Vermiculite	41.74
GMWC 100%	Grape manufacture waste compost	8.69
GMWC+CP (1:1)	Grape manufacture waste compost + Coco peat	17.39
GMWC+V (1:1)	Grape manufacture waste compost + Vermiculite	16.52
GMWC+CP+V (1:1:1)	Grape manufacture waste compost + Coco peat + Vermiculite	18.61
FWC 100%	Farm wastes compost	15.65
FWC+CP (1:1)	Farm wastes compost + Coco peat	20.87
FWC+V (1:1)	Farm wastes compost + Vermiculite	18.26
FWC+CP+V (1:1:1)	Farm wastes compost +Coco peat + Vermiculite	20.87
GMWC + FWC + CP + V (1:1:1:1)	Grape manufacture waste compost + Farm wastes compost + Coco peat + Vermiculite	17.83

20 days after sowing. Emergence was delayed in some substrates, therefore the velocity of emergence was calculated as mean days of emergence in each medium at the time period was set to 12 days after the date of first emergence in given in plot (Kahn et al., 2005).

At the end of seedlings growth period grew in nursery after 30 days from sowing, on April 14th and April 30th in the first and second experiment, respectively. Data recorded were: seedling height (it was measured from the media surface to the shoot apex); No. of leaves per seedling (excluding cotyledons); total pigments (the SPAD color reading -501, a portable leaf chlorophyll meter, Minolta corp.) was used for greenness measurements (Marquard & Tipton, 1987; Tenga et al., 1989 and Yadava, 1986) on fully expanded and apical leaves without destroying them, and fresh and dry weight of shoots and roots.

Statistical analysis was done by MSTAT statistic program and significant differences among the treatment means were assessed by multiple ANOVA (F and Duncan's multiple range tests) with a probability level of 95% (Sendecor & Cochran, 1980)

Results and discussion

Media characteristics

The composts differed from the coco peat in every measured variable (Table 2). FWC substrate displayed high electrical conductivity (EC) and pH than in other substrate, and the lowest EC and pH recorded in coco peat substrate. Organic matter content was over 50% in coco peat, and below 50% in GMC and FWC substrates; in contrast, total N in GMWC and FWC substrate more than doubled that of coco peat. Phosphorus and potassium content also were exceeded in coco peat and vermiculite, respectively.

The C/N ratio is widely used as an indicator of the maturity and stability of organic matter. The low value recorded here for the C/N ratio in GMWC, this suggests that the substrate was stable and mature. Davidson et al. (1994) reported that composts with a C/N ratio of less than 20 are ideal for nursery plant production. Ratios above 30 may be toxic causing plant death (Zucconi et al., 1981). Wilson et al. (2001) found that an increased proportion of compost in crop substrates prompted a decline in the C/N ratio compared to peats, although this will depend on the proportion of each ingredient in the mixture. From this appeared that the mixtures from GMWC with coco peat and vermiculite were suitable for seedling growth in nurseries.

Chemical characteristics (pH and EC) of substrate mixtures

The pH of GMWC medium (100%) was slightly acid and tended to moderate (6.74), while that of all other substrate mixtures was basic; the FWC medium displayed the highest pH value (8.71) (Figure 1). Substrate pH value in GMWC containing mixtures gives pH similar to CP + V mixture.

Although there is no ideal growth medium for nursery-produced horticultural crops (Poole et al., 1981; Raviv et al., 1986; Bugbee, 1996; Herrera et al., 2008), most greenhouse-grown species display better growth at slight acid pH values (5.2–7.0); GMWC medium and its mixtures approach from these values compared with FWC medium and its mixtures.

When rearing seedlings in the trays, salinity could be a adverse factor when it exceeded 100–300 ms/m (Gajdos, 1997). The GMWC medium had an EC of 1.87 dSm⁻¹, if we compared with FWC medium which had a higher EC value 7.66 dSm⁻¹ (Figure 1).

As for EC, the highest initial substrate values were recorded for mixtures containing FWC. The only substrates displaying the optimum EC values (<3.5 dSm⁻¹) recommended by Lemaire et al. (1985) and Wright (1986) for the growth and development of healthy, as well as vigorous seedlings are those containing GMWC substrate as a coco peat, which differed from the FWC substrate.

From that, the high pH and EC values of FWC substrate was far from the optimal pH and EC range for growing media, so that substrates should have low salinity because roots develop directly in them and this not achieve in FWC mixtures.

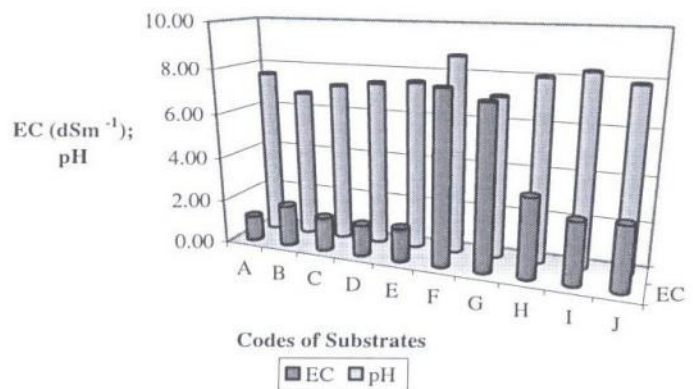


Figure 1 pH and EC values in the different mixtures of substrates (ten media) at the start of experiment. Codes: A = CP + V (1:1 v/v); B = GMWC 100%; C = GMWC + CP (1:1 v/v); D = GMWC + V (1:1 v/v); E = GMWC + CP + V (1:1:1 v/v/v); F = FWC 100%; G = FWC + CP (1:1 v/v); H = FWC + V (1:1 v/v); I = FWC + CP + V (1:1:1 v/v/v); J = GMWC + FWC + CP + V (1:1:1:1 v/v/v/v)

Table 2 Chemical characteristics of the initial substrates

Substrate	pH	EC	O.M %	O.C %	N %	P ppm	K ppm	C/N
Fresh GMW	8.21	2.37	71.3	41.5	1.3	–	–	31.8: 1
GMWC	6.74	1.87	49.6	28.1	1.81	678.0	20514	15.9: 1
FWC	8.71	7.66	39.8	23.1	1.41	594.9	776.8	16.5: 1
CP	4.69	1.57	53.2	30.3	0.65	966.0	7183.8	43.5: 1
V	7.9	0.76	–	–	0.002	39.0	86173.6	–

Table 3 Effect of different media on seedling establishment of tomato and cockscomb seeds in trays in greenhouse

Media (V/V)	Tomato				Cockscomb			
	Mean days to emergence		Emergence (%)		Mean days to emergence		Emergence (%)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	sExpt. 2
CP + V (1: 1)	5.76	5.9	94.3	97.0	6.74	6.91	93.5	96.3
GMWC 100%	6.43	6.81	92.3	93.4	7.2	6.92	91.3	90.8
GMWC + CP (1: 1)	6.04	6.24	94.5	97.0	6.03	6.40	92.56	93.4
GMWC + V (1: 1)	7.44	8.1	80.6	91.3	8.35	7.9	87.6	85.4
GMWC + CP + V (1: 1: 1)	5.91	5.94	96.4	98.5	6.85	7.19	96.2	95.8
FWC 100%	11.1	11.6	1.0	3.0	—	—	—	—
FWC + CP (1: 1)	10.9	—	4.9	—	9.4	11.3	33.1	10.0
FWC + V (1: 1)	10.1	11.2	20.8	25.0	9.81	10.5	8.1	40.3
FWC + CP + V (1: 1: 1)	8.84	9.3	50.7	63.2	7.23	7.10	61.4	68.5
GMWC + FWC + CP + V (1: 1: 1: 1)	7.06	8.1	74.6	78.8	7.01	7.5	80.4	75.5

Seedling emergence

Data presented in Table 3 showed that the control medium (CP + V) and media containing GMWC a decline in the mean days to seedlings emergence in two experiments of both tomato and cockscomb when compared with FWC and its mixtures. There was a marked delay in emergence rate 11.1 and 11.6 in the first and second experiments, respectively (over 5 days longer than CP + V and GMWC media in two experiments) of tomato and no emergence was appeared as cockscomb in FWC 100%.

Also, the highest emergence percentages were 96.4 and 98.5% in the first and second experiments, respectively were for of GMWC + CP + V medium of tomato and 96.2-96.3% in the first and second experiments for GMWC + CP + V and CP + V media, respectively of cockscomb (Table 3). While, the lowest emergence percentage zero – 1.0% was appeared in the first and second experiments, respectively of tomato and zero % in two experiments of cockscomb.

Increased pH and EC in substrates prompted a decline in the seedlings establishment rate and the higher seedlings emergence percentage was found for substrates with lower pH and EC values (Herrera et al., 2008). Burger et al. (1997) and Pinamonti et al. (1997) reported that low pH and EC values improve conditions for seedlings emergence in nurseries. The same behavior was recorded for all substrate mixtures studied; the fastest emergence rate and higher seeding emergence percentage was found for GMWC and its mixtures with lower pH and EC values.

Seedling growth

The growth of seedling (seedling height, No. of leaves, total pigments, root length and shoot and root fresh and dry weight) were significantly greater in GMWC + CP + VB medium than in the other substrate mixture of tomato in two experiments, and in the first experiment of cockscomb, but in the second experiment all traits were highly significant with the exception of shoot and root fresh weights and shoot dry weight (Figure 2, Tables 4 and 5). In contrast the seedlings grown in media comprised a FWC had little leaves, were

shorter and lighter in shoot and dry weights. the substrates GMWC, always produced the same seedlings appearance or tended to be more efficient for growth than the control medium (CP + V).

However, the visual color and quality of the plants suffered when plants were grown in 100% compost (waste yard trimmings) and its mixtures, also plants grown in compost based media had similar or lower shoot weights than the plants grown in peat based media (Wilson et al., 2002). Negative effects such as reduced seedling height

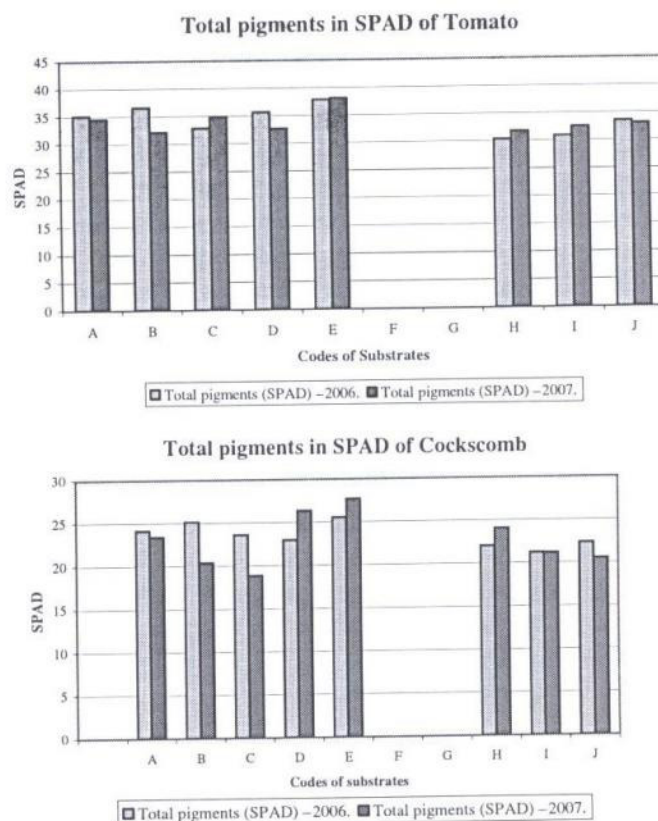


Figure 2 Effect of different media on total pigment of tomato and cockscomb seedlings during two experiments. Codes: A = CP + V (1:1 v/v); B = GMWC 100%; C = GMWC + CP (1:1 v/v); D = GMWC + V (1:1 v/v); E = GMWC + CP + V (1:1:1 v/v/v); F = FWC 100%; G = FWC + CP (1:1 v/v); H = FWC + V (1:1 v/v); I = FWC + CP + V (1:1:1 v/v/v); J = GMWC + FWC + CP + V (1:1:1:1 v/v/v/v).

began to appear with 40% compost (waste yard trimmings) and were generally apparent when compost comprised a majority of the medium (Kahn et al., 2005). The most likely explanation was the increase in EC and pH resulting from increments of FWC in the media had negative effects on seedling growth of tomato and cockscomb. From this growth increase indicated that this GMWC substrate improved the media characteristics, which seemed to provide better growth conditions for two plants than FWC substrate.

Battilani et al. (2003) reported that tomato root growth under different soil and climate conditions related to water and nutrient contents of medium. It is known that organic materials mixed with soil have a positive effect on plant growth as soil amendment and nutrient source (Eghball et al., 2004 and Westerman & Bicudo, 2005). So, GMWC substrate consider as organic material and source of many nutrients, which improve the growth of tomato and cockscomb seedlings.

Garcia-Gomez et al. (2002) found the lower weight of the calendula plants grown on peat compared with two composts might have been due to lower availability of macronutrients. Wang (1994) found that 'coarse-grade kenaf' media produced taller *Pittosporum tobira* plants compared to the commercial media tested. Abad et al. (2002) reported that high C/N ratio could cause immobilization of soluble nitrogen when coir dust was used as a growing medium for containerized plant production.

Conclusions

As waste products recycling is the main objective of the present work. The use of a mixture of GMWC with coco peat and vermiculite seemed to be the best medium in the nurseries of vegetable and ornamental plants to ensure a good seedling growth. Considering the use of compost in substrate preparation, the pH and EC were the main factors which can limit its use.

Table 4 Effect of different media on vegetative growth of tomato seedlings during two experiments

Media (V/V)	Seedling height (cm)	No. of leaves/seedling	Root length (cm)	Shoot fresh weight/seedling (g)	Root fresh weight/seedling (g)	Shoot dry weight/seedling (g)	Root dry weight/seedling (g)
Expt. 1							
CP + V (1: 1)	16.17 b	4.1 b	9.40 b	2.57 b	0.748 b	0.288 b	0.096 a
GMWC 100%	12.87 c	4.1 b	11.17 a	1.84 d	0.167 e	0.171 e	0.020 b
GMWC + CP (1: 1)	12.03 cd	4.1 b	11.03 a	2.04 c	0.303 c	0.278 c	0.031 b
GMWC + V (1: 1)	11.00 d	3.7 b	9.17 b	1.63 e	0.276 c	0.162 f	0.02 b
GMWC + CP + V (1: 1: 1)	19.47 a	4.8 a	10.93 a	3.06 a	0.998 a	0.321 a	0.076 a
FWC 100%	—	—	—	—	—	—	—
FWC + CP (1: 1)	—	—	—	—	—	—	—
FWC + V (1: 1)	6.07 e	2.3 c	4.47 d	0.51 g	0.055 g	0.053 h	0.034 b
FWC + CP + V (1: 1: 1)	7.53 e	2.4 c	7.30 c	0.67 f	0.115 f	0.057 g	0.01 b
GMWC + FWC + CP + V (1: 1: 1: 1)	13.03 c	4.1 d	8.37 d	1.79 d	0.238 d	0.173 d	0.022 b
F-test	**	**	**	**	**	**	**
Expt. 2							
CP + V (1: 1)	14.58 a	4.0 a	7.42 b	1.89 a	0.257 b	0.144 ab	0.037 b
GMWC 100%	10.6 c	3.5 b	6.42 bc	0.86 d	0.064 f	0.064 bc	0.021 e
GMWC + CP (1: 1)	10.17 c	3.5 b	7.25 b	0.94 cd	0.139 d	0.081 abc	0.025 d
GMWC + V (1: 1)	12.22 b	3.5 b	6.67 bc	1.32 b	0.197 c	0.106 abc	0.033 c
GMWC + CP + V (1: 1: 1)	14.18 a	4.17 a	9.25 a	1.87 a	0.391 a	0.152 a	0.051 a
FWC 100%	—	—	—	—	—	—	—
FWC + CP (1: 1)	—	—	—	—	—	—	—
FWC + V (1: 1)	6.75 d	2.67 cd	3.17 d	0.43 e	0.036 h	0.032 c	0.001 g
FWC + CP + V (1: 1: 1)	6.75 d	2.5 d	2.37 d	0.55 e	0.037 g	0.039 c	0.001 g
GMWC + FWC + CP + V (1: 1: 1: 1)	10.67 c	3.0 c	5.92 c	1.15 bc	0.114 e	0.079 abc	0.017 f
F-test	**	**	**	**	**	**	**

Values followed by the same letter in the same column are not significantly at the 95% level according to Duncan's test.

Table 5 Effect of different media on vegetative growth of cockscomb during two experiments

Media (V/V)	Seedling height (cm)	No. of leaves/seedling	Root length (cm)	Shoot fresh weight/seedling (g)	Root fresh weight/seedling (g)	Shoot dry weight/seedling (g)	Root dry weight/seedling (g)
Expt. 1							
CP + V (1: 1)	17.30 b	9.80 bc	8.37 b	3.28 b	0.734 b	0.288 b	0.078 b
GMWC 100%	7.97 f	8.37 d	8.4 b	1.81 e	0.19 e	0.169 e	0.018 f
GMWC + CP (1: 1)	13.93 c	10.07 b	7.7 bc	2.78 c	0.403 d	0.249 c	0.043 d
GMWC + V (1: 1)	9.97 e	9.7 bc	9.63 a	2.32 d	0.42 c	0.205 d	0.044 c
GMWC + CP + V (1: 1: 1)	20.30 a	12.13 a	9.3 a	4.46 a	0.990 a	0.414 a	0.100 a
FWC 100%	—	—	—	—	—	—	—
FWC + CP (1: 1)	—	—	—	—	—	—	—
FWC + V (1: 1)	5.47 h	6.57 e	5.7 d	0.69 h	0.087 h	0.073 h	0.004 h
FWC + CP + V (1: 1: 1)	6.67 g	7.37 e	7.17 c	0.85 g	0.113 g	0.078 g	0.013 g
GMWC + FWC + CP + V (1: 1: 1: 1)	11.13 d	9.10 cd	8.13 b	1.44 f	0.170 f	0.123 f	0.019 e
F-test	**	**	**	**	**	**	**
Expt. 2							
CP + V (1: 1)	11.0 bc	9.0 a	7.0 cd	1.41 c	0.241 e	0.133 a	0.030 c
GMWC 100%	8.58 d	9.67 a	7.23 bc	1.29 c	0.243 d	0.081 f	0.026 d
GMWC + CP (1: 1)	11.38 b	9.50 a	8.33 b	2.02 a	0.563 a	0.129 b	0.072 b
GMWC + V (1: 1)	10.5 c	9.17 a	5.92 de	1.41 c	0.259 c	0.099 d	0.02 e
GMWC + CP + V (1: 1: 1)	12.33 a	9.83 a	10.33 a	1.69 b	0.546 b	0.121 c	0.075 a
FWC 100%	—	—	—	—	—	—	—
FWC + CP (1: 1)	—	—	—	—	—	—	—
FWC + V (1: 1)	5.0 g	5.0 c	3.63 fg	0.38 e	0.029 h	0.025 h	0.001 g
FWC + CP + V (1: 1: 1)	6.25 f	7.50 b	2.5 g	0.89 d	0.048 g	0.065 g	0.001 g
GMWC + FWC + CP + V (1: 1: 1: 1)	7.22 e	8.17 b	4.83 e	1.26 c	0.174 f	0.091 e	0.019 f
F-test	**	**	**	**	**	**	**

Values followed by the same letter in the same column are not significantly at the 95% level according to Duncan's test.

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