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Perlite-An Effective Soilless Substrate for Producing Strawberry Plants Free from Nematode Transmitted Viruses

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A B S T R A C T

Strawberry (Fragaria *x ananassa* Duch.) is an important small-fruit crop, belonging to the family Rosaceae. In Himachal Pradesh, its cultivation under controlled environment is slowly gaining momentum but non-availability of good quality healthy planting material is a major obstacle in its commercial cultivation. Additionally, soil borne diseases, particular viruses transmitted by nematode vectors, cause severe production losses in strawberry culture. Use of soilless substrates can reduce the soil borne diseases besides improving the quality of the produce. The present studies were conducted at Horticultural Research & Training Station and Krishi Vigyan Kendra (HRTS & KVK) Kandaghat, Solan (H.P.) to evaluate different combinations of soilless media viz., perlite and cocopeat for preventing the spread of viruses transmitted through nematodes particularly *Strawberry Latent Ringspot Virus* (SLRSV), *Tobacco Ringspot Virus* (TRSV) and *Raspberry Ringspot Virus* (RRSV). Double antibody sandwich (DAS)-ELISA tests were performed to detect the presence of these nepoviruses in strawberry plants grown on soilless substrates. Results obtained in DAS-ELISA tests confirmed that plants grown under perlite were found to be free from viruses based on the O.D values measured at A_{405}nm in Microscan plate reader MS 5608A whereas the plants raised in soil tested positive for SLRSV, TRSV and RRSV. These findings indicate that soilless substrates can be used for producing strawberry plants free from nematode transmitted viruses.

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is an important small-fruit crop belonging to the family Rosaceae. It is an attractive fruit having a distinct pleasant aroma with delicate flavour and is a rich source of vitamins and minerals (Sharma, 2002). Because of its known flavour and vitamin contents, it is used regularly as part of the diet by many people around the world (Hancock, 1999). In Himachal Pradesh, it is being grown under open field condition on a limited scale in Kullu, Kangra, Sirmour, Solan and Shimla district over an area of 55 hectares with an annual production of 559 MT (Anonymous, 2017). Under controlled environment, its cultivation is slowly gaining momentum but, non-availability of good and healthy planting material creates a hindrance for its commercial cultivation. Further, soil

borne diseases particularly nematode transmitted nepoviruses cause severe production losses in strawberry culture. Generally, an ideal rooting media combination can provide sufficient porosity, aeration and water holding capacity which can enhance crop growth and productivity but, soilless media combinations in particular, can also reduce the soil borne diseases and prevent the spread of nematode transmitted viruses. Keeping this in view, it was thought worthwhile to carry out the investigation with the following objectives:

The main objectives of this study to standardize the growing media for producing good quality planting material (elite runners) and fruits under protected conditions and also to study the possible role of soilless substrates in preventing the spread of nepoviruses in strawberry.

Materials and Methods

The present investigation was carried out at the Horticultural Research & Training Station and Krishi Vigyan Kendra (HRTS & KVK) Kandaghat, Solan (H.P.) under a polyhouse having side and top ventilation and equipped with sprinkler and drip irrigation system. Elite plants of strawberry cv. Chandler were planted in 1 x 1 m beds filled with different soilless substrate combinations at a distance of 20 x 20 cm in the second week of October, 2016 (Plate 1). Plants were irrigated using sprinkler irrigation and were fertilized using soluble fertilizer (19:19:19) through drip irrigation system. The Experiment was laid out in a Completely Randomised Block Design using the following treatments:

T_1: Perlite
T_2: Perlite + FYM (1:1)
T_3: Cocopeat
T_4: Cocopeat + FYM (1:1)
T_5: Perlite + Cocopeat + FYM (1:1:1)
T_6: Soil + FYM (1:1)
T_7: Soil (Control)
Replications: 4

Collection of samples

Ten plants per treatment were randomly marked for recording the data on fruit and runner parameters. out of these fiv/e strawberry plants in each treatment were marked and leaves were collected from the marked plants and brought to the laboratory in ice bucket for conducting DAS (Double Antibody Sandwich) -ELISA tests as per the protocol given by Clark and Adams (1977).

DAS-ELISA

Wells of the microtitre plate (BIOREBA, Switzerland certified microplates) except those of the top and bottom rows and rows on the extreme left and right, were filled with 200µl aliquots of coating antibodies diluted in 1x coating buffer (1:1000 ratio v/v). The plate was incubated in humid box for 4 hours at 30° C. The coating antibody suspension was removed by shaking out the plate over the wash basin. The wells were filled with 1x PBS-Tween and kept for 2 minutes with gentle shaking. The plate was emptied and filled again with PBS-Tween. The washing was repeated three times. The test samples were grounded in 1x extraction buffer (1:10 ratio v/v). All coated wells were filled with 200µl aliquots of test samples (each sample in duplicate) besides positive and negative control wells. The plate was incubated in humid box overnight at 4±1° C. The washing steps were repeated as mentioned above. Alkaline phosphate (ALP) conjugated antibodies were filled in each well with 200µl aliquots after diluting it in 1x ECI (enzyme conjugated immunoglobin) buffer at a (ratio of 1:1000 v/v).

Table.1 DAS-ELISA detection for nepoviruses

Antibody	Locality	Media	Mean OD value(at 405nm)		
SLRSV	Kandaghat	Perlite	Test Sample	Positive Control	Negative Control
		Perlite+FYM(1:1)	0.078(-)	0.236(+)	0.056(-)
		Cocopeat	0.092(-)		
		Cocopeat+FYM(1:1)	0.150(+)		
		Perlite+Cocopeat+FYM(1:1:1)	0.161(+)		
		Soil+FYM	0.165(+)		
		Soil(Control)	0.177(+)		
			0.187(+)		
TRSV	Kandaghat	Perlite	Test Sample	Positive Control	Negative Control
		Perlite+FYM(1:1)	0.082(-)	0.170(+)	0.047(-)
		Cocopeat	0.095(-)		
		Cocopeat+FYM(1:1)	0.178(+)		
		Perlite+Cocopeat+FYM(1:1:1)	0.185(+)		
		Soil+FYM	0.227(+)		
		Soil(Control)	0.232(+)		
			0.244(+)		
RRSV	Kandaghat	Perlite	Test Sample	Positive Control	Negative Control
		Perlite+FYM(1:1)	0.069(-)	0.138(+)	0.046(-)
		Cocopeat	0.082(-)		
		Cocopeat+FYM(1:1)	0.304(+)		
		Perlite+Cocopeat+FYM(1:1:1)	0.325(+)		
		Soil+FYM	0.376(+)		
		Soil(Control)	0.377(+)		
			0.398(+)		

Table.2 Effect of different growing media on number of runners, root length and berry yield in strawberry cv. Chandler

Treatments	Number of runners	Root length (cm)	Yield per plant (g)	Yield per ha (t/ha)
	Pooled	Pooled	Pooled	Pooled
T_1 Perlite	39.25	19.13	201.39	50.35
T_2 Perlite + FYM (1:1)	40.00	19.16	203.32	50.83
T_3 Cocopeat	34.75	16.70	189.14	47.29
T_4 Cocopeat + FYM (1:1)	34.50	16.72	190.52	47.63
T_5 Perlite + Cocopeat + FYM (1:1:1)	36.25	17.85	196.64	49.16
T_6 Soil + FYM(1:1)	31.25	14.88	185.11	46.28
T_7 Soil	27.85	10.90	151.36	37.84
$CD_{0.05}$	1.14	2.80	0.79	0.19

Table.3 Effect of different growing media on berry weight, size and TSS in strawberry cv. Chandler

Treatment	Berry weight (g)	Berry length (mm)	Berry breadth (mm)	Total soluble solids (%)
	Pooled	Pooled	Pooled	Pooled
T_1 Perlite	21.21	37.51	24.79	10.09
T_2 Perlite + FYM (1:1)	21.62	38.01	25.55	10.52
T_3 Cocopeat	18.52	33.38	22.78	9.37
T_4 Cocopeat + FYM (1:1)	18.93	34.00	23.64	9.49
T_5 Perlite + Cocopeat + FYM (1:1:1)	20.47	36.04	23.88	9.82
T_6 Soil + FYM (1:1)	17.45	31.53	21.57	8.50
T_7 Soil	16.00	27.08	19.82	7.76
$CD_{(0.05)}$	0.62	1.40	0.57	0.54

Fig.1 ELISA plate showing positive reaction with SLRSV, TRSV & RRSV

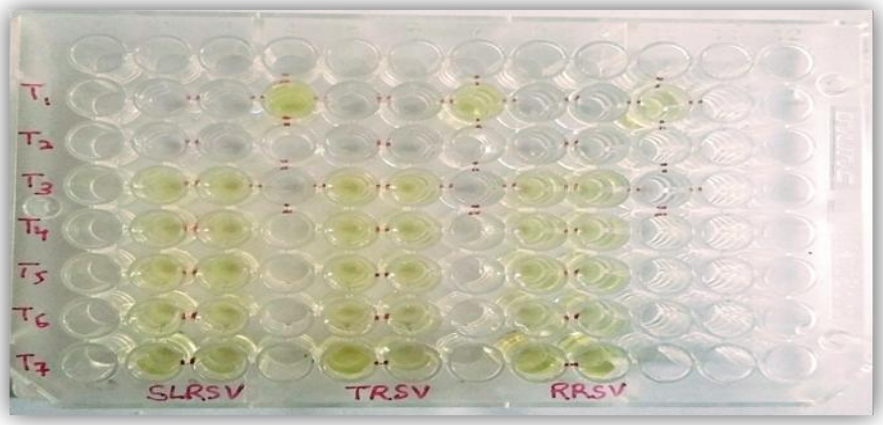

Fig.2 Root length of healthy plants

Fig.3 Planting of strawberry plants under different soilless media

Fig.4 Fruiting under T1: Perlite

The plate was incubated in humid box for 5 hours at 30° C. The washing was done as mentioned above. p-nitrophenyl phosphate (pNPP) substrate was dissolved in 1x substrate buffer by dissolving 5mg pNPP tablet in 5ml of 1x substrate buffer. Each well was filled with 200µl aliquots of the substrate. The plate was kept in humid box in the dark condition at room temperature until a yellow colour was clearly visible in the positive control (usually between 30-60 minutes). The results were assessed either by visual observations or by measurement of the absorbance value of the hydrolysed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre plate reader (Micro Scan MS 5605A, Electronics Corporation of India Limited, Hyderabad). The results of ELISA for the detection were interpreted as per Dijkstra and Jager (1998) as samples were considered infected when their absorbance values (A_{405nm}) exceeded two times the mean values of respective healthy control samples.

Results and Discussion

A perusal of data presented in Table 1 indicates the presence of all three viruses in all treatments except for perlite and perlite + FYM. Results obtained in DAS-ELISA tests confirmed that plants grown under perlite were found to be free from viruses based upon the OD values measured at A_{405}nm. It is clear from these results that perlite can help in producing strawberry plants free from the nematode transmitted viruses.

Data presented in Table 2 reveals that perlite and perlite + FYM proved to be the best media for strawberry cultivation resulting in healthier plants with higher fruit yield and better runner development. The treatments that tested negative for the viruses were also found to be the best in respect of plant growth and fruit characters (Table 3) thereby clearly indicating that virus indexed plants grown in soilless substrates particularly perlite and perlite + FYM were healthier and had superior horticultural traits. The positive influence of perlite and its mixtures on better root development may have improved aeration thus forming greater root system which may have promoted shoot nutrient uptake leading to increased berry yield. These findings are in conformity with the findings of a number of workers (Ghazvani *et al.,* 2007; Jafarnia *et al.,* 2010; Hassan *et al.,* 2011). Better berry weight, size and TSS in plants grown under perlite and perlite + FYM treatment as observed in Table 3 may beattributed to the ability of this medium to provide essential micro nutrients to the plants and improve the nutrient availability due to better features of the growing media (Fig. 2–4).

Symptoms of mixed infection were exhibited by strawberry plants grown in different media at kandaghat thus making it virtually impossible to recognize the virus on the basis of visual indexing. Hence, different isolates of strawberry were serologically indexed after visual indexing based on the symptoms observed and it was confirmed that plants grown under perlite were found to be free from viruses whereas the plants raised in soil tested positive for SLRSV, TRSV and RRSV. Therefore, ELISA proved to be a handy and reliable tool for proper identification and characterization of these viruses (Fig. 1). These studies will help in the producing strawberry plants free from nematode transmitted viruses with better yield of good quality fruits and healthy runners.

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