



Influence of Sowing Media and Variety on Growth Parameters and Disease Incidence of Early Cauliflower (*Brassica oleracea var. botrytis*) Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Healthy seedling production is prerequisite for raising vigorous and profitable crops. The present investigation was carried out at Assam Agricultural University, Jorhat, Assam in the year 2018 and 2019 to access the impact of sowing media and variety on growth and incidence of disease in

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nursery. The treatments consisted of four different sowing media [M_1 -cocopeat (60): vermiculite (20): perlite (20), M_2 -cocopeat (50): vermicompost (50), M_3 -cocopeat (50): vermicompost (50): microbial consortium and M_4 -Conventional nursery] and two varieties [V_1 (White Diamond) and V_2 (CFL1522)]. Thus, 8 treatment combinations were laid out in a 4x2 factorial arrangement in a Randomized Completely Block Design (RCBD) with three replications. The treatment M_1 , M_2 and M_3 were inside green house. The results revealed that seedlings raised in different sowing media surpassed the conventional sowing media for growth attributes and disease incidence. The highest seedling stem diameter (0.50 cm), fresh weight (5.44 g) and highest dry weight (0.64 g) was recorded by seedlings grown in M_2 i.e cocopeat(50):vermicompost (50), while M_3 -cocopeat (50): vermicompost (50): microbial consortium recorded maximum seedling growth index (1469.86). The lowest value for all the growth parameters were recorded in conventional nursery(M_4). Non-significant effect of variety was noticed for seedling stem diameter. Variety V_1 (White Diamond) registered maximum seedling fresh weight (4.12 g), dry weight (0.43 g) and seedling growth index (1254.49). The least disease incidence (2.36 %) was recorded in M_3 i.e cocopeat (50): vermicompost (50): microbial consortium and in variety V_2 (CFL1522) with 4.76 %. The interaction effect was also found to be significant for growth parameter as well as for disease incidence. The treatment combination M_2V_1 recorded the maximum seedling stem diameter (0.53 cm), fresh weight (5.50 g) and dry weight (0.66 g) while M_3V_2 was found to be superior than all other treatment combination with maximum seedling growth index (1482.03) and with the lowest disease incidence (2.11 %). Since M_3 contains the component of M_2 and resulted the lowest incidence of disease which is an important criteria of quality seedlings and considering the superiority of V_1 with respect to most of the growth parameters it can be concluded that for producing good quality seedlings of early cauliflower under protected condition the treatment combination M_3V_1 can be used .

Keywords: Cauliflower; sowing media; seedling; growth; disease.

1. INTRODUCTION

Cauliflower (*Brassica oleracea L. var. botrytis*) belongs to family Brassicaceae and is popular for its white tender head or curd. Cauliflower is one of the most important winter season vegetables of India which is rich source of protein, carbohydrates, vitamin-B and C as well as various minerals which are necessary for the human health. The edible flowering head known as 'curd' is used as fried vegetable, dried vegetable, for making soups, and pickles. In Assam, early Cauliflower is sown during mid-July to first week of August which matures by the month of October. The crop is gaining commercial status in Assam, provides the farmers with a substantial return as it is available in market early in the season during October.

"Recently, the use of high quality seedlings produced in facilities where climatic conditions are kept under control has increased. In the production of ready-to-plant seedlings, climatic conditions as well as seed sowing media have quite significant impact. Seedling production with conventional methods causes stress in plants. Seeds are sown in different media, which plays a vital role in efficient production of horticultural

seedlings in nurseries" [1]. "The use of suitable growing media or substrates for sowing of seeds directly affects the germination, development of functional shoot and root system. A good growing medium provides sufficient anchorage or support to the plant, serves as reservoir for nutrients and water, allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate" [2].

The soil is generally used as a basic medium because it is cheapest and easily available. Supplementing the soil with organic matter (vermicompost, cocopeat) and inorganic amendment like vermiculite, perlite is common practice so as to make it more porous and enrich with adequate nutrients for the seedlings. Essentially perlite and vermiculite are used in the horticultural industry because they both provide good aeration and drainage, they can retain and hold substantial amount of water and later release it as per the requirement. These are sterile and free from diseases, having a fairly neutral pH (especially perlite which is neutral), non-toxic, safe to use, and relatively inexpensive. Perlite is a grey-white siliceous material of volcanic origin mined from lava flows. It is very

light (6 to 8 lbs/cubic feet), chemically inert and odourless. The high processing temperature results in a sterile product. Water will adhere to the surface of the perlite but is not absorbed into the perlite aggregates. It has negligible cation exchange capacity.

Vermiculite is a micaceous mineral, which expands markedly when heated. Chemically, it is hydrated magnesium-aluminium-iron silicate and very light in weight (6 to 10 lbs/cubic feet). It absorbs large quantities of water and also holds positively charged nutrients like potassium, magnesium and calcium because of its plate like structure.

“Cocopeat or coir is an agricultural by-product obtained after the extraction of fibre from the coconut husk [2]. Cocopeat is considered a growing medium component with acceptable pH, EC and other chemical attributes [2] as it has good physical properties, high total pore space, high water content, low shrinkage, low bulk density and slow biodegradation [3,4], significant amount of phosphorous (6-60 ppm) and potassium (170-600 ppm) and can hold water up to nine times its weight. Since it contains more lignin and less content of cellulose than peat, it is more resistant to microbial breakdown, and therefore, may shrink less and also easily re-wet after drying”.

“Likewise, vermicompost refers to a mixture of worm casting, organic material, humus, living earthworms, their cocoons and other organisms. Vermicompost, an odorless peat-like substance has good structure, moisture-holding capacity, relatively large amounts of available nutrients, and microbial metabolites that may act as plant growth regulators. Soil provides natural support to plant, sand provides proper aeration in media, cocopeat and vermiculite gives warm conditions, high water holding capacity and vermicompost as a source of organic manure provides better nutrition to the germinating seedlings” [5].

A pre-sowing inoculation of planting material as well as the planting medium with the consortia of beneficial microorganisms is an innovative approach for production of quality and healthy seedlings in horticultural production in general and transplanted vegetables in particular. A microbial consortium is a carrier based product containing nitrogen fixing, phosphorus and potassium solubilising and plant growth promoting microorganisms in a single

formulation. The synergistic effect of the formulated microbes can help in providing healthy and vigorous seedlings and considerably reducing the cost of cultivation by reducing fertilizer requirement of vegetables.

Generally in conventional nursery (soil, sand and FYM) damping off diseases in seedling causes extensive loss to the growers. Since no systematic research work on effect of growing media and variety on quality seedling production of early cauliflower under green house in Jorhat condition the present investigation has been undertaken.

2. MATERIALS AND METHODS

The experimental site was conducted in the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat (26.47 °N latitude and 94.12 °E longitude and at 86.8 m above MSL) during the month of July-August for two consecutive years, 2018 and 2019. The experiment composed of four nursery media composition [M₁- Cocopeat (60): Vermiculite (20): Perlite (20); M₂-Cocopeat (50): Vermicompost (50); M₃-Cocopeat (50): Vermicompost (50): Microbial consortium and M₄: Conventional nursery (soil: sand: FYM)] and two variety (V₁- White Diamond and V₂ - CFL 1522). Thus, eight treatment combinations were laid out in a 4x2 factorial arrangement in a Randomized Completely Block Design (RCBD) with three replications. The pro trays were used for nursery raising with cocopeat, vermiculite, perlite, vermicompost and microbial consortium as growing media and seeds of early cauliflower hybrid variety ‘White Diamond’ and ‘CFL-1522’ which are popular and commercially available in Jorhat were sown in pro trays (one seed per cell) under green house in the second week of July. One conventional nursery was also raised for sowing same seeds at same time with soil, sand and FYM as growing media. Microbial consortium consisting of *Azotobacter*, *Azospirillum* and Phosphate Solubilising Bacteria (PSB) was mixed with respective media at a ratio of 1:100 and mixed properly and sprinkled and heaped. Coco peat was soaked and washed before mixing with other media.

Different seedling growth attributes namely seedling stem diameter, fresh weight, seedling dry weight and seedling growth index were recorded just before transplanting. The

stem diameter was measured using a vernier caliper of six sample seedlings from each treatments and average was computed and expressed in centimeter. These seedlings were again used for verification of fresh weight of seedlings. Fresh weight of seedlings was recorded in an electronic balance and average weight (gram) was computed. The sample seedlings from each treatment which were used for verification of fresh weight of seedlings were subsequently placed in butter paper bag and dried for 72 h in a hot air oven maintained at 60 °C. The dried seedlings were removed and kept in desiccators to record the weight in an electronic balance and expressed in gram.

Seedling Growth index (SGI) was computed from the germination (%) and total seedling length i.e. root length + shoots length as suggested by [6] and expressed as whole number.

$SGI = \text{Germination (\%)} \times (\text{Shoot length} + \text{Root length})$

For recording disease incidence, the number of seedlings as affected by diseases mainly pre and Post damping off were recorded and expressed as per cent incidence.

Pooled data of two years was taken (2018 & 2019) for drawing conclusions after subjecting the same to statistical analysis using the statistical package SPSS (20.0) at 5% critical difference (CD) for testing the significant differences among the treatment means.

3. RESULTS AND DISCUSSION

3.1 Effect of Sowing Media and Variety on Growth Parameters

3.1.1 Seedling stem diameter

The data presented in Table 1 reveals significant effect of sowing media on stem diameter in both the years and in pooled analysis. In the year 2018, 2019 and in pooled analysis maximum diameter (0.48, 0.53 and 0.50 cm) was recorded in M_2 which was followed by M_3 and M_1 and minimum (0.21, 0.23 and 0.22 cm) was

recorded in M_4 which was statistically at par with M_1 . Non-significant effect of cauliflower variety was observed in the year 2018 and in pooled analysis though it was significant in the year 2019.

Significant interaction effects were observed for stem diameter. In both the years and pooled analysis, M_2V_1 registered maximum stem diameter (0.50, 0.57 and 0.53 cm) which, however, did not differ significantly from M_2V_2 . The minimum stem diameter (0.20 cm) was obtained in M_1V_2 and M_4V_1 but was at par with M_1V_1 and M_4V_2 in 2018. In the year 2019 and in pooled analysis also, M_1V_2 recorded the minimum stem diameter (0.20 cm) but was not significantly lower than M_1V_1 , M_4V_1 and M_4V_2 . Higher stem diameter of seedlings is an advantage as they are known to resist transplanting shock. Maximum stem diameter obtained in M_2 (cocopeat and vermicompost) might be due to better nutrient availability leading to higher production of photosynthetically functional leaves in these treatments finally resulting in better diameter of seedling. This indicates that the positive growth effects of vermicompost might be due to the additional nutrients, especially NO_3-N provided by the vermicompost. Alternatively, vermicompost might have increased the cation exchange capacity (CEC) of the media, which allowed it to retain more positively charged nutrients, especially NH_4^+ . This is in agreement with the findings of [7] in sweet pepper transplant, [8] in tomato, [9] in pepper and brinjal transplants and [10] in tomato seedlings. Combined influence of M_2 with variety V_1 produced maximum stem diameter.

3.1.2 Seedling fresh weight

Data presented in Table 1 revealed that seedling fresh weight was significantly affected by different sowing media and variety. In both the years and pooled analysis, M_2 (cocopeat and vermicompost) recorded the maximum fresh weight (5.43, 5.45 and 5.44 g) which was statistically superior to all other media. Minimum fresh weight of 2.69, 2.67 and 2.68 g was recorded in the treatment M_4 . Variety 'White Diamond' i.e. V_1 exhibited higher fresh weight (4.12 g) than V_2 than in both the years and pooled analysis.

Table 1. Effect of sowing media, variety and their interaction on seedling stem diameter, fresh weight, dry weight and growth index of cauliflower seedling

Media (M)	Seedling stem diameter (cm)			Seedling fresh weight (g)			Seedling dry weight (g)			Seedling growth index		
	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
M ₁	0.22	0.24	0.23	3.17	3.04	3.11	0.32	0.34	0.33	1135.87	1131.84	1133.85
M ₂	0.48	0.53	0.50	5.43	5.45	5.44	0.64	0.65	0.64	1277.38	1279.40	1278.39
M ₃	0.28	0.33	0.30	3.97	4.02	3.99	0.39	0.39	0.39	1470.44	1469.27	1469.86
M ₄	0.21	0.23	0.22	2.69	2.67	2.68	0.26	0.25	0.25	1026.79	1026.45	1026.62
S.Ed.(±)	0.03	0.03	0.02	0.20	0.22	0.13	0.01	0.02	0.01	44.59	43.13	27.76
CD(0.05)	0.07	0.06	0.04	0.43	0.47	0.27	0.02	0.03	0.02	95.65	92.51	56.35

Variety(V)

V ₁	0.30	0.36	0.33	4.12	4.12	4.12	0.43	0.44	0.43	1254.40	1254.58	1254.49
V ₂	0.29	0.30	0.30	3.51	3.47	3.49	0.37	0.38	0.37	1200.83	1198.91	1199.87
S.Ed.(±)	0.02	0.02	0.01	0.14	0.15	0.09	0.01	0.01	0.01	31.53	30.50	19.63
CD(0.05)	NS	0.04	NS	0.31	0.33	0.19	0.01	0.02	0.01	NS	NS	39.85

Interaction (MXV)

T ₁ (M ₁ V ₁)	0.23	0.27	0.25	3.79	3.76	3.78	0.40	0.41	0.40	1173.13	1174.45	1173.79
T ₂ (M ₁ V ₂)	0.20	0.20	0.20	2.55	2.32	2.44	0.25	0.26	0.26	1098.60	1089.23	1093.91
T ₃ (M ₂ V ₁)	0.50	0.57	0.53	5.51	5.49	5.50	0.65	0.66	0.66	1345.41	1346.44	1345.92
T ₄ (M ₂ V ₂)	0.45	0.48	0.47	5.35	5.40	5.38	0.62	0.64	0.63	1209.36	1212.36	1210.86
T ₅ (M ₃ V ₁)	0.27	0.35	0.31	4.52	4.57	4.55	0.44	0.45	0.45	1457.18	1458.18	1457.68
T ₆ (M ₃ V ₂)	0.30	0.30	0.30	3.42	3.46	3.44	0.34	0.33	0.33	1483.70	1480.37	1482.03
T ₇ (M ₄ V ₁)	0.20	0.23	0.22	2.67	2.65	2.66	0.24	0.23	0.23	1041.90	1039.23	1040.57
T ₈ (M ₄ V ₂)	0.22	0.22	0.22	2.71	2.69	2.70	0.28	0.27	0.28	1011.67	1013.67	1012.67
S.Ed.(±)	0.04	0.04	0.03	0.28	0.31	0.19	0.01	0.02	0.01	63.06	60.99	39.26
CD(0.05)	0.09	0.09	0.06	0.61	0.66	0.39	0.03	0.04	0.02	135.27	130.83	79.69

M₁ - Cocopeat: Vermiculite : Perlite @3:1:1; M₂- Cocopeat: Vermicompost@1:1 ; M₃-Cocopeat:Vermicompost (1:1): Microbial consortium @ 1: 100; M₄- Soil: Sand: FYM , V₁ - White Diamond, V₂ -CFL 1522

Table 2. Effect of sowing media, variety and their interaction on disease incidence (%) of cauliflower seedling

Media (M)	Disease incidence (%)		
	2018	2019	Pooled
M ₁	4.72	4.78	4.75
M ₂	3.22	3.22	3.22
M ₃	2.44	2.28	2.36
M ₄	10.33	8.94	9.64
S.Ed.(±)	0.48	0.55	0.27
CD(0.05)	1.04	1.19	0.54

Variety(V)			
V ₁	5.30	5.14	5.22
V ₂	5.05	4.47	4.76
S.Ed.(±)	0.68	0.78	0.19
CD(0.05)	NS	NS	0.38

Interaction (MXV)			
T ₁ (M ₁ V ₁)	5.44	5.22	5.33
T ₂ (M ₁ V ₂)	4.00	4.33	4.16
T ₃ (M ₂ V ₁)	3.44	3.44	3.44
T ₄ (M ₂ V ₂)	3.00	3.00	3.00
T ₅ (M ₃ V ₁)	2.77	2.44	2.61
T ₆ (M ₃ V ₂)	2.11	2.11	2.11
T ₇ (M ₄ V ₁)	9.55	9.44	9.50
T ₈ (M ₄ V ₂)	11.11	8.44	9.78
S.Ed.(±)	0.34	0.39	0.38
CD(0.05)	0.73	0.84	0.77

M₁- Cocopeat: Vermiculite : Perlite @3:1:1; M₂ - Cocopeat: Vermicompost @1:1 ; M₃- Cocopeat: Vermicompost(1:1): Microbial consortium @ 1: 100; M₄- Soil: Sand: FYM ; V₁-White Diamond; V₂ - CFL 1522

Increased fresh weight in M₂ was due to the effect of vermicompost on plant growth and development were not only nutritional but can be attributed to hormonal and biochemical effects. [11] also observed that vermicompost increased the leaf area and biomass in plants. Increased N levels are known to enhance vegetative growth and chlorophyll content in a manner similar to vermicompost supplementation. Results obtained was in accordance with the results of [7,8,12]. Maximum seedling fresh weight in V₁ might be its genetical character.

The interaction effect of media and variety also showed significant effect on fresh weight. Maximum fresh weight (5.51, 5.49 and 5.50 g) was observed in M₂V₁ in 2018, 2019 and pooled data, respectively which was statistically at par with M₂V₂, immediately followed by M₃V₁ and

M₁V₁. The minimum fresh weight (2.55, 2.32 and 2.44 g) was recorded in M₁V₂.

Combined influence of M₂ with variety V₁ showed significant effect on seedling fresh weight was due to the synergistic combination of both factors in improving the physical conditions of the media and nutritional factors with the better genetical makeup of variety.

3.1.3 Seedling dry weight

It is evident from the Table 1 that there were significant differences among sowing media and between the two varieties for seedling dry weight. The highest dry weight (0.64, 0.65 and 0.64 g) was observed in M₂ followed by M₃ and M₁ in 2018, 2019 and in pooled analysis, respectively. The lowest dry weight (0.26, 0.25 and 0.25 g) was recorded in the treatment M₄.

During both the years and pooled analysis, V_1 (White Diamond) resulted higher dry weight (0.43, 0.44 and 0.43 g) than V_2 (CFL 1522).

The interaction effects were found to be significant. Maximum dry weight (0.65, 0.66 and 0.66 g) was observed in both the years and pooled data in M_2V_1 but was not statistically different from M_2V_2 and which was followed by M_3V_1 and M_1V_1 . The minimum dry weight (0.24, 0.23 and 0.23 g) was recorded in M_4V_1 which, however, was at par with M_1V_2 (0.25 and 0.26 g) in both the years but was significantly higher in pooled analysis (0.26 g).

Increased seedling dry weight might be due to favorable conditions for better growth of the seedling, particularly for good development of a root system leading to absorption of more nutrients and thus produced seedlings with more growth of leaves and increased the photosynthesis which led to increase fresh weight of seedlings and there by dry weight of seedlings. As because the hormone production is affected by the nutritious food, so vermicompost with high nutrient content resulted in the increase of growth stimulus hormones and consequently resulted in the dry matter increase [13]. This result is also in conformity with that of [7] and [14]. The positive impact of vermicompost as growth media on increased dry weight of chilli and brinjal transplants was also highlighted by [9].

3.1.4 Seedling growth index

A perusal of data presented in Table 1 showed that sowing media had a profound effect on seedling growth index. The highest seedling growth index (1470.44 and 1469.27) was recorded in M_3 which was statistically superior to next higher seedling growth index (1277.38 and 1279.40) which in turn differed significantly from other treatments in the year 2018 and 2019. In the pooled analysis also, M_3 registered highest seedling growth index (1469.86) and proved statistically superior to other treatments. The lowest seedling growth index (1026.79, 1026.45 and 1026.62) was registered in M_4 in both the years and pooled analysis, respectively.

The effect of variety on seedling growth index was found to be non-significant in both the years but in the pooled data over two years showed a significant variation between the two varieties. The seedlings of variety White Diamond (V_1)

exhibited higher growth index (1254.49) over V_2 (1199.87).

Significant interaction effects among different treatments were observed in both the years of study and pooled mean. Maximum growth index (1483.70, 1480.37 and 1482.03) was recorded in M_3V_2 and minimum growth index (1011.67, 1013.67 and 1012.67) was recorded in M_4V_2 .

“The humic acid and humic substances of vermicompost might have enhanced the soil physical condition and helped in availability, solubility, mobility and utilization of plant nutrients resulting in enhanced seedling growth at early stage” [15]. “Seedling inoculation with biofertilizer have enhanced the efficacy of the vermicompost and improved the seedling growth attributes over uninoculated treatments. This can be attributed to reason that production of plant growth promoting substances by plant growth-promoting microbes which were known to cause, higher nitrogen fixation by nitrogen fixing bacteria, phosphorous solubilization by phosphorous solubilizing bacterium increased the availability of nitrogen and phosphorous that might have led to enhanced cell division and better root development, uptake and transportation of water and nutrients and resulted in enhanced seedling growth. On the other hand, cocopeat provide better aeration for root growth and add phosphorous and potassium to the growing seedlings. This finding is in agreement with” [16]. “Since the seedlings are pre-colonized with the efficient microbes, they are likely to survive better and put forth good growth and yields. When two or three organisms are inoculated together, they are known to show better performance over single inoculations, possibly due to their synergistic interaction” [17,18] Besides, the inoculation of mixed cultures of microorganisms with different functional properties is known to enrich the microbial diversity in the rhizosphere of crop plants and thereby enhance microbial interactions.

3.2 Effect of Sowing Media And Variety On Disease Incidence (%) Of Seedlings

The data pertaining to the effect of sowing media and variety on disease incidence, mainly damping off disease of seedling, have been presented in Table 2. The sowing media had significant influence on disease incidence percent in both the years and pooled data.

The lowest disease incidence (2.44 and 2.28 %) was recorded in M_3 in 2018 and 2019. However, it was at par with M_2 (3.22 %) in both the years and was followed by M_1 (4.72 and 4.78 %). The highest disease incidence (10.33 and 8.94 %) was recorded in conventional media with soil, sand and FYM (M_4) in both the years. In pooled analysis, M_3 exhibited the similar trend with lowest disease incidence (2.36 %) which was followed by M_2 (3.22 %) and the highest incidence (9.64%) was recorded in M_4 .

The effect of variety was found to be non-significant in both the years but pooled analysis resulted significant differences and V_2 exhibited minimum disease incidence (4.76 %) while V_1 recorded maximum (5.22 %) incidence.

The interaction between media and variety was found to be statistically significant during the years and pooled data. In both the years, M_3V_2 recorded minimum disease incidence (2.11 %) which was at par with M_3V_1 (2.77 and 2.44 %) and followed by M_2V_2 (3.00 %). The maximum incidence (11.11 %) was observed in M_4V_2 in 2018 and in M_4V_1 (9.44 %) in the year 2019. In the pooled data, minimum incidence (2.11 %) was observed in M_3V_2 , which was at par with M_3V_1 (2.61 %) and the maximum incidence (9.78 %) was observed in M_4V_2 but not statistically different from M_4V_1 (9.44 %)

Reduced incidence of disease at seedling stage might be due to healthy and vigorous growth of seedlings with vermicompost and biofertilizer along with cocopeat (M_3). This finding is in conformity with [19] who reported that "apart from quality seedling production, organic amendments also reduced damping off disease in seedling stage of cabbage". "Consortia of beneficial microbes in the rhizosphere is reported to act synergistically by stimulating each other through physical and/or biochemical process and provides essential nutrients to plants and protect them from disease causing microbes" [20].

In the interaction (M_3V_2) the lowest incidence might be due to the synergistic action of media and the variety having genetically resistance to disease.

4. CONCLUSION

From the study it can be concluded that sowing media and variety had a substantial impact on

seedling stem diameter, fresh and dry weight, seedling growth index and incidence of disease. The sowing media cocopeat (50) : vermicompost (50) and variety white Diamond had maximum seedling stem diameter, fresh and dry weight while cocopeat (50): vermicompost (50): microbial consortium (1:100) with variety CFL-1522 recorded highest seedling growth index and least damping off disease incidence in seedling stage under green house. Since M_3 contains the component of M_2 and resulted the lowest incidence of disease which is an important criteria of quality seedlings and considering the superiority of V_1 with respect to most of the growth parameters it can be concluded that for producing good quality seedlings of early cauliflower under protected condition the treatment combination M_3V_1 can be used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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