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Studies on different formulations of organic growth promoter for mulberry cuttings

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Abstract
Organic growth promoters plays an important role in building up soil fertility, increasing the moisture holding capacity and enhancing the growth of micro-organisms in the soil. Mulberry prefers almost neutral soil reaction for its luxuriant growth. Organic fertiliser is rich in nitrogen, phosphorus, potassium, calcium, and other plant nutrition elements as well as organic material, which has a positive effect on soil properties. The study aimed to determine the effect of organic growth promoters with different formulations on the growth and development of mulberry cuttings. Stem cuttings of *Morus indica* were treated with pellets (20, 40, 50 and 60 g/plant), topical application (20, 40, 50 and 60 ml/plant) and soil application (20, 40, 50 and 60 ml/plant). The experiment was conducted with 4 replications, and it follows Factorial Completely Randomised Design (FCRD). The data collected from the experiment were statistically analysed using AGRESS at a probability level of 5 per cent. Results showed that, among all the treatments, growth parameters such as highest rooting percentage (75%), high survival rate (90%), longest root length (53.29 cm), huge microbial population ($79x10^6$), highest plant height (77.72 cm), widest leaf area (173.01 cm^2) and highest number of leaves (23.50) were observed in the cuttings treated with T_1P_1 (20 g pellet/plant). It is concluded that organic growth promoter in pellet form at a rate of 20 g/plant highly improves both the root and shoot parameters of mulberry cuttings and also increases the microbial population in the rhizosphere region. These growth promoter pellets can be recommended for producing good quality mulberry saplings.

Keywords: Mulberry, nursery, organic growth promoter, pellets, stem cuttings

Introduction
Mulberry, the sole food plant of silkworm (*Bombyx mori* L.) is a perennial crop cultivated for more than 15- 20 years in the same land and it is a prime constituent of the sericulture industry (Chakraborty *et al.*, 2016) [1]. The current sericulture industry demands new varieties suitable for various agro-climatic conditions. Suitable parent material needs to be identified from a large number of germplasm genotypes for this purpose. Besides, estimates of genetic diversity and the relationship between various collections from diverse origins help inefficient management and utilization of germplasm (Tulu *et al.*, 2022) [2]. The continuous production of mulberry for a long time results in gradual reduction of leaf yield and quality. The highly intensive mulberry cropping system causes depletion of nutrients in soil and excessive usage of inorganic fertilizers as well as pesticides results in deleterious effect on soil health. Even though inorganic fertilizers add necessary nutrients to the soil, their regular use causes long-term depletion of organic matter, soil compaction and degradation of overall soil quality. Organic farming is considered to be an alternative agricultural practice to mitigate the adverse effects of various inorganic fertilizers to soil conditions. This alternative practice is expected to improve the sericulture industry and the production of quality mulberry leaves. Mulberry prefers almost neutral soil reaction for its luxuriant growth. Organic fertilizer is rich in nitrogen, phosphorus, potassium, calcium, and other plant nutrition elements as well as organic material, which has a positive effect on soil properties. Organic fertiliser can change the microbial environment in the soil, increase the activity of soil enzymes, improve the root development and absorption capacity of plants at the seedling stage, activate the soil nutrient system, change microbial flora, reduce soil bulk density, and can also improve the disease resistance of plants, reduce diseases, promote crop development, and can significantly increase crop yield and improve crop quality (Yao *et al.*,

2024, Nandini *et al*., 2024) [3, 4]. Comparing the effect on crop yield, the nutrients contained in manure are slightly behind compared to the same quantity of mineral fertilizers (Pocius *et al*., 2017) [5]. However, nutrients contained in manure have organic form, and therefore are less washed out of soil, are released into soil gradually without resulting in high concentrations of salts. This not only increases the yield, but also the quality of crops (increased quantities of vitamins, sugar, protein, starch, and no nitrite accumulation). Given moisture shortage in soil, granules release the moisture gradually thus creating the best conditions for plant roots and microorganisms. Organic manure plays an important role in building up soil fertility and increasing moisture holding capacity and growth of micro-organisms in the soil. The introduction of crop benefiting microbial inoculants into soil play a significant role in the mobilization of various nutrients needed by the crop. Application of microbial inoculants in conjunction with organic manures has significantly increased the productivity of mulberry leaf (Mary *et al*., 2015) [6]. The main requirement imposed to newly developed organic granulated fertilizers is the possibility to use them under specific conditions. The new fertilizer pellets developed on the basis of organic compost are attributed to anisotropic materials. Keeping this in view, an attempt has been made to study the effect of organic pellets on the improvement in the growth parameters of mulberry plants (*Morus indica*), which is the aim of the present investigation.

Materials and Methods

A field experiment was carried out in the mulberry garden, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam located at 11.20° North latitude and 76.56° East longitude at an altitude of 320 m above mean sea level.

Treatment details: The following are the treatment details.

Treatment	Organic formulation
T_1P_1	20 g pellet/plant
T_1P_2	40 g pellet/plant
T_1P_3	50 g pellet/plant
T_1P_4	60 g pellet/plant
T_1P_5	Control
T_2F_1	20 ml topical application/plant
T_2F_2	40 ml topical application/plant
T_2F_3	50 ml topical application/plant
T_2F_4	60 ml topical application/plant
T_2F_5	Control
T_3S_1	20 ml soil application/plant
T_3S_2	40 ml soil application/plant
T_3S_3	50 ml soil application/plant
T_3S_4	60 ml soil application/plant
T_3S_5	Control

2.1 Preparation of Organic Growth Promoter

2.1.1 Liquid formulation: The ingredients such as yeast extract (20 g/l), beef extract (20g/l), peptone (20g/l), finely ground bone meal powder (20g/l) and agar (1g/l) were mixed thoroughly. After sterilization, citric acid (30 g/l) was added as a preservative. A mixture of 50 g beeswax and 2g of borax was added at a rate of 100ml/l as emulsifier. For masking the bad smell, Dalchini powder was added at a rate of 12g/l.

2.1.2 Pelletization: Pellets were formed by using lignite as carrier material and guar gum as binding agent in the ratio of 39:1. The carrier and binder are mixed thoroughly by adding the organic growth promoter and the pellets were prepared with the use of a pelletizer.

After 75 days of planting, parameters such as rooting percentage, survival rate, microbial count, plant height, number of leaves and leaf area were recorded in all the treatments.

2.2 Experimental design and statistical analysis: The experiment was conducted with 4 replications and it follows Factorial Completely Randomized Design (FCRD). The data collected from the experiment were statistically analyzed using AGRESS by adopting the standard procedure outlined by Panse and Sukhatme (1978) [7] at a probability level of 5 percent.

Results and Discussion

The effect of treatments on rooting percentage is shown in Table 1. The highest rooting percentage was found in cuttings treated with 20 g pellets/plant (75%) followed by 40ml soil application/plant and 40 ml topical application/plant with the rooting percentage of 72.5% and 70%, respectively and control had the lowest rooting of 45%. The presence of auxins has initiated root growth and improved root functionality. This is in line with Kotis *et al.*, (2009) [8] who stated that exogenous auxin plays a major role in increasing the rooting ability. This was strengthened by Wiesman and Lavee (1995) [9] who said that the organic products or treatments applied to the basal end of the cuttings improved rooting capacity. Similar results were found by Murthy and Yadav (1969) [10] who concluded that growth regulators are used to improve rootability. Similar studies were made by El-Leithy *et al*., (2006) [11] in *Salvia officinalis* and Mady (2009) [12] in faba bean.

Table 1: Effect of the organic formulations on rooting per cent and survival rate

Treatments	Rooting per cent (%)	Survival rate (%)
T_1P_1	75.00	90.00
T_1P_2	57.50	77.50
T_1P_3	50.00	57.50
T_1P_4	47.50	52.50
T_1P_5	45.00	37.50
T_2F_1	52.50	62.50
T_2F_2	70.00	85.00
T_2F_3	62.50	77.50
T_2F_4	55.00	67.50
T_2F_5	45.00	37.50
T_3S_1	60.00	70.00
T_3S_2	72.50	87.50
T_3S_3	67.50	82.50
T_3S_4	65.00	72.50
T_3S_5	45.00	37.50
Mean	58.00	66.33
SE(d)	0.85	0.82
CD (0.05)	1.71	1.66

The effect of organic formulations on the survival rate is shown in Table 1. Maximum survival rate was found in 20 g pellets/plant (90%) followed by 40ml soil application/plant and 40 ml topical application/plant with 87.5% and 85%, respectively while control showed poor survivability of 37.5%. This is due to application of organic growth promoter indicate the cutting ability to assimilate nutrients, survive in the soil, have structural support, and develop buds to ensure the future CO_2 assimilation of the plant, these also indicate the acclimatization for future planting, which may increase survival efforts. This was supported by Hawramee *et al.,* (2019) [13] and Zengingbal and Esitken (2016) [14]. Similar results were found by Lokanath and Shivashankar (1986) [15] in mulberry, Arancon and Edwards (2005) [16], Nikiema *et al.,* (2013) [17] and Boiago *et al.,* (2019) [18] in crambe seeds.

The effect of organic formulations on microbial count is shown in Table 2. On 75 DAP bacterial count was maximum in 20g pellet/plant (1.89 log cfu/g) followed by 40ml soil application/plant (1.81 log cfu/g) and 40 ml topical application/plant (1.77 log cfu/g) while control has lowest population of 1 log cfu/g. On 75 DAP, the fungal count was maximum in 20g pellet/plant (1.52 log cfu/g) followed by 40ml soil application/plant (1.43 log cfu/g) and 40 ml topical application/plant (1.39 log cfu/g) and control has lowest population of 0.77 log cfu/g.On 75 DAP, the actinomycetes count was maximum in 20g pellet/plant (2.21 log cfu/g) followed by 40ml soil application/plant (2.12 log cfu/g) and 40 ml topical application/plant (2.07 log cfu/g) whereas control had the lowest population of 1.64 log cfu/g). These results were supported by El-Hak *et al.,* (2012) [19] who stated that indirect effects of pellets on improved status of soil fertility which would have led to improve the functional ability. Also this is in line with the statement that organic amendments are frequently used to improve the soil structure, microbial diversity and plant nutrient status (Sun *et al.,* 2014 and Ling *et al.,* 2016) [20, 21]. This was further strengthened by Ji *et al.,* (2017) [22] who reported that pellets stimulated N mineralization process, increased in available nutrient content of rhizospheric soil after application of OGP which has been attributed to improved diversity of microbial community.

Table 2: Effect of the organic formulations on microbial population on 75th day

Treatments	Microbial population (log cfu/g)		
	Bacteria	Fungi	Actinomycetes
T_1P_1	1.89	1.52	2.21
T_1P_2	1.73	1.36	1.94
T_1P_3	1.20	0.90	1.72
T_1P_4	1.17	0.85	1.66
T_1P_5	1.00	0.77	1.64
T_2F_1	1.51	1.08	1.75
T_2F_2	1.77	1.39	2.07
T_2F_3	1.63	1.28	1.91
T_2F_4	1.60	1.18	1.81
T_2F_5	1.00	0.77	1.64
T_3S_1	1.58	1.20	1.79
T_3S_2	1.81	1.43	2.12
T_3S_3	1.65	1.27	1.92
T_3S_4	1.61	1.25	1.89
T_3S_5	1.00	0.77	1.64
Mean	1.48	1.13	1.85
SE(d)	0.027	0.018	0.03
CD (0.05)	0.055	0.036	0.05

The effect of organic formulations on plant height on different days after planting is shown in Table 3. Maximum plant height was observed in 20 g pellets/plant (77.72 cm) followed by 40ml soil application/plant and 40 ml topical application/plant with a height of 74.45 cm and 68.95 cm respectively while control had minimum plant height of 36.80 cm. This may be due to increased callus induction response which was higher due to PGPR application. This is in accordance with Nelson (2004) who stated that PGPR is able to exert a beneficial effect on plant growth and PGPR might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients facilitating the uptake of nutrients by the plants (Burd *et al.*,2000) [24].

This was further highlighted by Ahmed *et al.*, (2017) [25] who reported that pellets contain most of the plant nutrients which increases some of the soil properties such as organic matter, S, Zn and P content in soil which may improve soil texture, water holding capacity, soil aggregation and soil moisture retention. The positive effects of PGPR on the growth and yield of crops such as wheat (Ozturk *et al.*, 2003 and Salanture *et al.*, 2006) [27], maize (Egamberdiyeva, 2007) [28], soybean (Cattelan *et al.*, 1999) [29] and were explained that by nitrogen fixing ability, phosphate solubilizing capacity and phytohormones production. Similar results were recorded by Tiwari *et al.*, (2003) [30] in onion and Ofori-Amanfo *et al.*, (2018) [31] in lettuce.

Table 3: Effect of the organic formulations on plant height on different days after planting

Treatments	Plant height (cm)			
	30th day	45th day	60th day	75th day
T_1P_1	21.27	33.58	52.30	77.72
T_1P_2	18.92	29.88	50.05	68.05
T_1P_3	18.81	29.25	45.95	59.38
T_1P_4	18.45	32.25	43.18	52.28
T_1P_5	17.38	22.63	28.65	36.80
T_2F_1	19.01	27.75	48.65	62.10
T_2F_2	20.37	32.63	48.73	68.95
T_2F_3	20.09	29.00	47.58	64.33
T_2F_4	19.56	31.58	45.33	63.35
T_2F_5	17.38	22.63	28.65	36.80
T_3S_1	19.18	30.18	48.63	63.15
T_3S_2	20.52	33.13	50.30	74.45
T_3S_3	20.14	32.00	48.73	65.50
T_3S_4	19.89	30.50	46.35	63.53
T_3S_5	17.38	22.63	28.65	36.80
Mean	19.22	29.31	44.12	59.55
SE(d)	0.28	0.37	0.50	0.86
CD (0.05)	0.56	0.75	1.01	1.73

The effect of organic formulations on leaf area on different days after planting is shown in Table 4. Maximum leaf area was observed in the treatment 20 g pellets/plant (173.01 cm^2) followed by 40ml soil application/plant and 40 ml topical application/plant with 162.47 cm^2 and 159.88 cm^2 respectively whereas control had minimum leaf area of 69.42 cm^2. This is because PGRs are involved in activating cell division and elongation in meristematic tissues. This is in accordance with Asghar *et al.*, (2002) [32] who reported that increase in the photosynthetic area and translocation of photosynthates which subsequently increased the formation of leaves with more area. This was highlighted by Prud'homme *et al.*, (1992) [33] who concluded that PGPR is able to exert a beneficial effect on plant growth which increases leaf area. Similar studies were done by Mady (2012) [34] who said that yeast extract might contribute to the influence on metabolism and their stimulating effect on photosynthetic pigments and enzyme activity which leads to increased vegetative growth of field bean. Similar results were observed by Sajid *et al.*, (2009) [35] in lily, Rawgol *et al.*, (2011) [36] in mulberry and Azizi and Mahmoudabadi (2013) [37] in sesame.

Table 4: Effect of the organic formulations on leaf area on different days after planting

Treatments	Leaf area (cm^2)			
	30th day	45th day	60th day	75th day
T_1P_1	55.73	91.26	133.15	173.01
T_1P_2	41.19	62.79	111.03	158.43
T_1P_3	33.42	37.45	59.76	118.32
T_1P_4	32.83	34.54	56.13	82.42
T_1P_5	23.12	26.84	39.99	69.42
T_2F_1	35.73	47.72	113.24	133.03
T_2F_2	51.57	69.23	120.03	159.88
T_2F_3	45.26	52.37	102.56	142.69
T_2F_4	38.48	50.26	101.28	135.73
T_2F_5	23.12	26.84	39.99	69.42
T_3S_1	36.50	48.39	99.84	133.21
T_3S_2	53.29	87.13	122.52	162.47
T_3S_3	48.62	58.61	117.01	151.14
T_3S_4	39.07	51.18	86.96	141.13
T_3S_5	23.12	26.84	39.99	69.42
Mean	38.74	51.43	89.57	126.65
SE(d)	0.50	0.70	1.09	1.58
CD (0.05)	1.01	1.40	2.19	3.19

The effect of organic formulations on the number of leaves on different days after planting is shown in Table 5. On 75 DAP more number of leaves was observed in 20 g pellets/plant (23.50) followed by 40ml soil application/plant and 40 ml topical application/plant with 18.75 and 16.25 leaves respectively while control had less number of leaves (11.50). This is because of increased cell division and cell expansion thereby increasing the number of leaves/plant. This is in line with the findings of Qian *et al.*, (2008) [38] in alfalfa, Selvakumar *et al.*, (2009) [39] in blackgram, Abbas (2013) [40] in *Vicia faba*, Ali and Mahmoud (2013) [41] in mungbeans, Toaima (2014) [42] in sage plants and Shafeek *et al.*, (2015) [43] in onion.

Table 5: Effect of the organic formulations on the number of leaves on different days after planting

Treatments	Number of leaves			
	30th day	45th day	60th day	75th day
T_1P_1	5.75	10.50	18.25	23.50
T_1P_2	2.75	7.75	12.25	15.75
T_1P_3	2.00	7.75	9.25	13.00
T_1P_4	1.75	7.00	10.75	12.50
T_1P_5	1.50	6.25	9.00	11.50
T_2F_1	2.25	8.00	11.50	13.75
T_2F_2	4.25	9.25	13.25	16.25
T_2F_3	3.50	7.50	11.25	15.25
T_2F_4	3.00	6.50	12.00	14.25
T_2F_5	1.50	6.25	9.00	11.50
T_3S_1	2.50	8.25	12.50	13.25
T_3S_2	4.75	9.25	13.75	18.75
T_3S_3	3.75	8.75	12.75	15.50
T_3S_4	3.25	7.25	11.00	14.00
T_3S_5	1.50	6.25	9.00	11.50
Mean	2.93	7.80	11.70	14.68
SE(d)	0.04	0.11	0.20	0.22
CD (0.05)	0.08	0.22	0.40	0.44

Conclusion

Based on the results obtained from the current study, it is concluded that organic growth promoter in pellet form at a rate of 20 g/plant highly improves both the root and shoot parameters of mulberry cuttings and also the increases the microbial population in the rhizosphere region. Presently, no such work has been done in mulberry. Hence, this study is found to be a promising method to produce good quality mulberry saplings. Also, the growth and development of mulberry saplings can be improved within the nursery period.

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