

## INTERACTION BETWEEN IBA, CERTAIN MICRO-NUTRIENTS AND PHENOLIC ACIDS IN RELATION TO ROOTING OF TEA CUTTINGS

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A study has been carried out to identify the micro-nutrients and phenolic compounds that are beneficial in rooting of tea cuttings; an attempt has also been made to identify their optimal dosage as well as their additive effect with IBA.

Phenolic compounds did not exert favourable influence on rooting either alone or with IBA or with a mixture of IBA and micro-nutrients. IBA and zinc at 5000 ppm each, applied separately, improved rooting and root growth characteristics.

A mixture of IBA and zinc at 5000 ppm each in powder formulation proved to be superior to the Rooting Hormonal Formulation based on IBA, that is being widely used now.

### INTRODUCTION

The use of synthetic auxins and hormone-like substances to promote rooting in tea clonal cuttings is known for a long time (Visser, 1958 ; Venkataramani, 1959 ; Haridas, 1975 ; Sharma, Satyanarayana and Haridas, 1981 ; Sharma, 1984). Several investigations revealed that indolebutyric acid (IBA), indoleacetic acid (IAA), naphthylacetic acid (NAA) and dimethylamine succinamic acid, either individually or in different combinations, induce early and profuse root system in tea cuttings (Sharma, 1984).

Formulations based on IBA, such as Seradix-B2\* are generally used to pretreat green, semi-hardwood cuttings. But, the response of tea cuttings to such formulations has, generally, been found to be indifferent, often resulting in excessive callusing. Investigations spanning over a period of eight years led to the development of a rooting hormonal formulation (RHF), specific to tea cuttings; this is a powder formulation based on IBA, with the pH of the carrier adjusted between 4.2 and 4.8 (Sharma, 1984). This formulation has proved to be significantly superior to the others that are now available in the market in advancing a higher per cent of rooting as well as the volume and dry weight of root system in tea cuttings.

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\* A product of M/s. May & Baker

There have been some interesting reports about the response of cuttings of some species to pretreatment with certain micro-nutrients like zinc and boron, either on their own or in combination with auxins (Venkataramani, 1962*a, b*; Sharma, 1974; Mohandass, Mani and Sampath, 1982). Certain phenolic compounds have also been known to be synergistic with auxins (Basu *et al.*, 1969; Bose, Roy and Basu, 1972; Rupa, Dhawan and Nanda, 1984; Kakkar and Rai, 1986). Such findings offer scope to refine RHF further or evolve another formulation that is more effective than RHF.

The present study was undertaken to identify the micro-nutrients and phenolic acids and their optimum dosage rates that are beneficial in the rooting of tea cuttings as well as, their additive effect with IBA. Results from such a study are likely to assist in further improving the efficacy of RHF.

### MATERIALS AND METHODS

Two experiments were carried out in the nursery of UPASI Tea Research Institute, S. India (ca 1060 m AMSL), the first from May to December 1985 and the second from October 1986 to May 1987. The treatments included in the first and second experiments are given below :

#### Treatments (ppm)

Experiment 1	Experiment 2
T Standard 1	T Standard 1
*T RHF 2	T IBA 5000 2
T IBA 10000 3	T Zinc 2500 3
T IBA 5000 4	T Zinc 5000 4
T O - HBA 10 5	T RHF <sup>o</sup> 5
T O - HBA 20 6	T Zinc 2500 * 6
T P - HBA 10 7	T Zinc 5000 * 7
T P - HBA 20 8	T IBA 5000 + Zinc 2500 8
T Zinc 5000 9	T IBA 5000 + Zinc 5000 9
T Boron 100 10	T IBA 5000 + Zinc 2500 * 10
T IBA 5000 + O - HBA 10 11	T IBA 5000 + Zinc 5000 * 11
T IBA 5000 + O - HBA 20 12	T IBA 5000 + O - HBA 20 12

T <sub>13</sub> IBA 5000 + P - HBA 10	T <sub>13</sub> IBA 5000 + P - HBA 10
T <sub>14</sub> IBA 5000 + P - HBA 20	T <sub>14</sub> IBA 5000 + Zinc 2500 + O - HBA 20
T <sub>15</sub> IBA 5000 + Boron 100	T <sub>15</sub> IBA 5000 + Zinc 5000 + O - HBA 20
T <sub>16</sub> IBA 5000 + Zinc 5000	T <sub>16</sub> IBA 5000 + Zinc 2500 + P - HBA 10
*T <sub>17</sub> IBA 5000 + Zinc 5000	T <sub>17</sub> IBA 5000 + Zinc 5000 + P - HBA 10
T <sub>18</sub> IBA 5000 + P - HBA 10 + Boron 100	
T <sub>19</sub> IBA 5000 + P - HBA 20 + Zinc 5000	
T <sub>20</sub> IBA 5000 + O - HBA 10 + Boron 100	
T <sub>21</sub> IBA 5000 + O - HBA 20 + Zinc 5000	

\*In powder formulation ; others in solution

More promising treatments from Experiment 1 and a few others were included in Experiment 2.

The experiments were of randomized block design, comprising three replicates of 100 cuttings each.

The clone, UPASI-17, was used in the first experiment and UPASI-2 and UPASI-8 in the second.

Boron at 100 ppm with boric acid as the source and zinc at 2500 and 5000 ppm with zinc sulphate as the source were used in the experiments. IBA was tried at 5000 and 10000 ppm and the phenolic acids, ortho-hydroxybenzoic acid and para-hydroxybenzoic acid at 10 and 20 ppm each ; the same concentrations were used in the mixtures as well.

Treatments 2 and 17 in the first experiment and 5, 6, 7, 10 and 11 in the second were imposed as powder formulations, while the remaining treatments were imposed in solution form.

The powder formulations were prepared according to the method developed by Sharma (unpublished). The solution formulations were prepared by dissolving the required quantity of the appropriate chemical in 50 per cent ethyl alcohol.

The treatments in solution formulations were applied by the quick-dip method (Eden, 1954) : the bottom cut-end of the cuttings were dipped in the respective solutions for five seconds before planting them for rooting. The other treatments were applied by dipping the bottom cut-end, up to about 3.00 mm, in the respective powder formulations prior to striking the cuttings for rooting. Cuttings dipped in water before planting served as standards for comparison.

After imposing the treatments, the cuttings were raised in the nursery according to standard practice (Sharma, 1976).

The percentage of rooting was determined at the end of 8 and 12 weeks, while biometric parameters were recorded at the end of 20 and 24 weeks in the first experiment. In the second experiment, observations were made at 4-weekly intervals from the 8th to the 24th week.

Ten cuttings, at random, were taken from each replicate for examination at the time of recording observations ; the cuttings were carefully uprooted, washed thoroughly and brought to the laboratory in sealed polythene covers.

Length of roots per cutting was recorded by totalling the length of all the roots ; dry weight of roots and shoots was determined after keeping the samples in an oven at 80°C, for 24 h.

## RESULTS AND DISCUSSION

IBA at 5000 ppm proved to be superior to 10,000 ppm and the standard in per cent rooting and certain important growth parameters ; IBA at 10,000 ppm was comparable to the standard in all respects except higher dry weight of roots (Figs 1,2). IBA at 5000 ppm in powder formulation (RHF) has been found to be superior to the same dosage in solution and the standard in all respects (Figs 1,2; Tables 1-5). The shoot weight, in general, has not been affected adversely by treatment with IBA.

Pretreatment of cuttings with IBA has been reported to supplement the endogenous level of auxin and increase its availability in the surface area of the cut-end, which in turn, assists in rhizogenesis (Audus, 1953). The involvement of auxin in the conversion of starch into simple transportable sugars, the accumulation of sugars at the treated surface and their progressive decline with the progress of rooting (Audus, 1953) suggest an important role to the applied auxin.

Powder formulations have, generally, evoked superior response over the corresponding treatments applied through solution. The superior performance of the powder formulations could be attributed to the pH of the carrier adjusted between 4.2 and 4.8 ; pH of the solution formulations was maintained at 6.0 and above. Auxins are said to be in an undissociated state at a pH of 4.0 to 5.0, resulting in a faster uptake; dissociation of auxins sets in at higher levels of pH leading to poorer uptake (Krishnamoorthy, 1981).

The phenolic compound, ortho-hydroxybenzoic acid, depressed rooting at both concentrations, upto eight weeks ; thereafter, it had no effect on per cent rooting (Fig. 1B). Para-hydroxybenzoic acid had no effect on per cent rooting. Biometric parameters of root growth were not altered by either of the two phenolic acids at the rates tried (Figs 1A, 2C, D and E) ; however, treatment with para-hydroxybenzoic

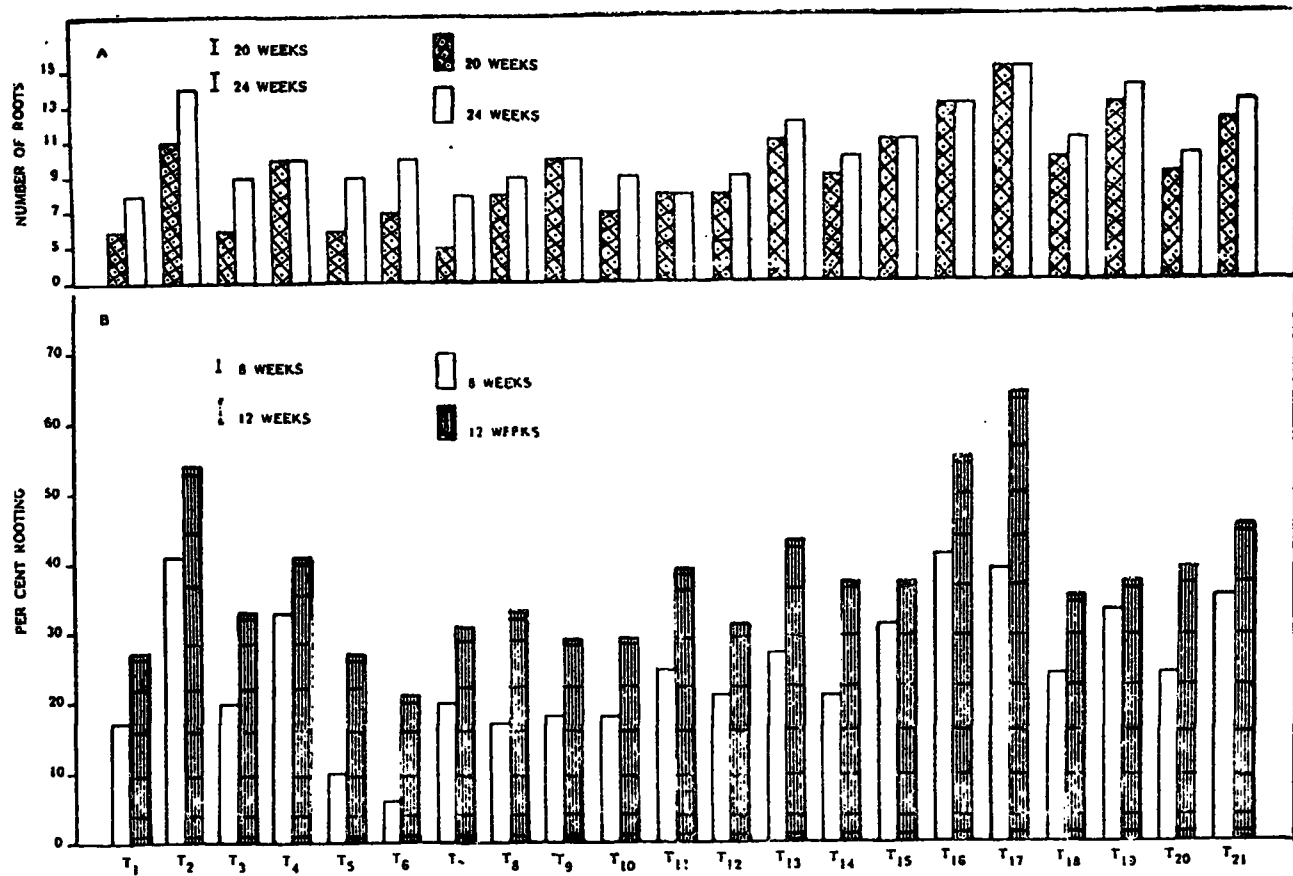


Fig. 1—Effect of treatments, on per cent rooting and on number of roots in UPASI - 17, Experiment 1.

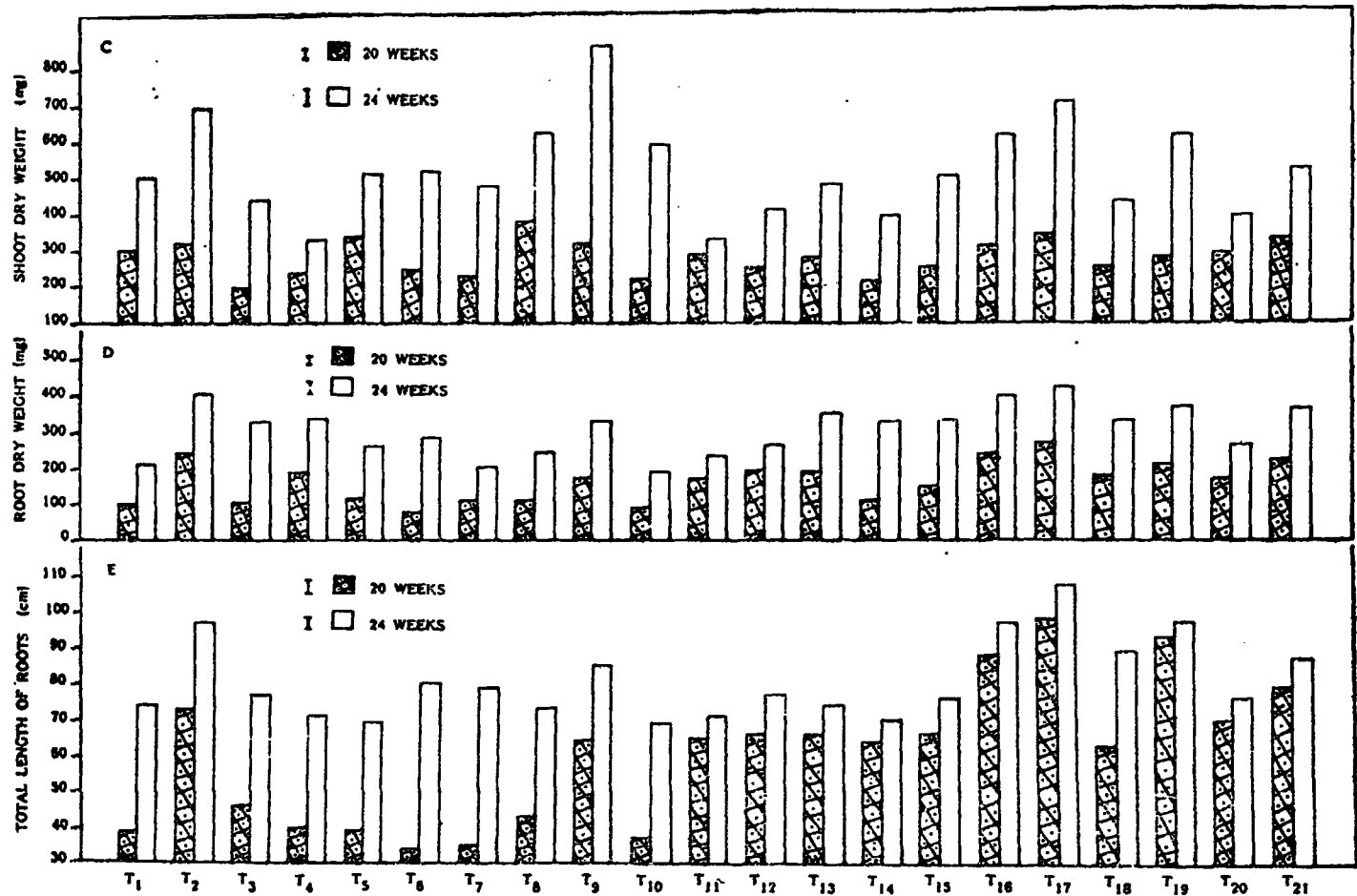


Fig. 2.—Effect of treatments on total length of roots and of root and Shoot dry weight in UPASI-17, Experiment 1.

TABLE I — Effect of treatments on percentage\* of rooting (Means of three replicates)  
Experiment 2

Treatments (ppm)	UPASI - 2					UPASI - 8				
	Weeks after planting					Weeks after planting				
	8	12	16	20	24	8	12	16	20	24
Standard	33	43	55	60	75	37	43	55	59	78
IBA 5000	39	49	63	68	90	43	49	59	69	90
Zinc 2500	30	43	49	63	87	35	47	51	59	84
Zinc 5000	31	47	51	68	90	37	55	57	64	90
RHF*	41	57	70	87	90	54	59	66	72	90
Zinc 2500*	39	57	63	66	87	45	55	59	64	84
Zinc 5000*	39	59	66	72	90	46	57	63	66	90
IBA 5000 + Zinc 2500	41	59	66	72	90	49	59	64	68	90
IBA 5000 + Zinc 5000	43	63	75	90	90	61	66	72	84	90
IBA 5000 + Zinc 2500*	43	63	72	87	90	55	64	72	84	90
IBA 5000 + Zinc 5000*	49	66	86	90	90	63	69	84	90	90
IBA 5000 + O-HBA 20	38	51	59	70	87	47	55	61	68	84
IBA 5000 + p-HBA 10	34	51	59	72	90	47	55	61	69	90
IBA 5000 + Zinc 2500 + O-HBA 20	38	53	61	72	90	53	57	66	68	90
IBA 5000 + Zinc 5000 + O-HBA 20...	34	55	63	68	90	49	59	66	72	90
IBA 5000 + Zinc 2500 + p-HBA 10	39	51	61	68	87	49	55	64	69	84
IBA 5000 + Zinc 5000 + p-HBA 10	39	53	61	79	90	49	57	63	72	84
CD (P = 0.05)	4	6	5	5	5	7	7	7	6	9
CV (%)	6	7	5	5	3	9	8	7	6	7

\* Arcsin transformed values.

Note : Treatments with an asterisk are in powder formulation , others in solution formulation.

TABLE 2—Effect of treatments on number of roots per cutting (Means of three replicates)  
Experiment 2

Treatments (ppm)	UPASI - 2					UPASI - 8				
	Weeks after planting					Weeks after planting				
	8	12	16	20	24	8	12	16	20	24
Standard	3	6	7	8	9	4	5	6	7	8
IBA 5000	5	8	9	10	10	5	6	7	8	9
Zinc 2500	3	6	7	8	8	4	5	5	7	7
Zinc 5000	4	7	8	9	9	5	5	6	7	8
RHF *	5	10	11	13	14	6	7	7	8	9
Zinc 2500 *	5	7	8	9	9	5	6	7	8	8
Zinc 5000 *	5	7	9	9	10	5	6	8	8	9
IBA 5000 + Zinc 2500	5	7	9	10	10	6	7	8	8	9
IBA 5000 + Zinc 5000	5	9	11	12	13	7	8	9	10	11
IBA 5000 + Zinc 2500 *	5	10	11	12	13	7	8	8	9	10
IBA 5000 + Zinc 5000 *	6	12	13	14	14	8	9	9	11	12
IBA 5000 + O-HBA 20	4	7	9	10	10	5	6	7	8	8
IBA 5000 + p-HBA 10	4	7	9	9	10	5	6	7	8	9
IBA 5000 + Zinc 2500 + O-HBA 20	4	6	9	10	10	6	6	7	9	9
IBA 5000 + Zinc 5000 + O-HBA 20	5	7	9	10	11	6	6	8	9	9
IBA 5000 + Zinc 2500 + p-HBA 10	5	7	9	10	10	6	6	7	9	9
IBA 5000 + Zinc 5000 + p-HBA 10	5	7	9	10	11	5	7	7	9	10
CD (P = 0.05)	1	1	1	1	2	1	1	1	1	1
CV (%)	10	10	9	7	9	9	8	9	9	8

Note : Treatments with an asterisk are in powder formulation, others in solution formulation.

TABLE 3—Effect of treatments on total length (cm) of roots per cutting (Means of three replicates)  
Experiment 2

Treatments (ppm)	UPASI - 2					UPASI - 8				
	Weeks after planting					Weeks after planting				
	8	12	16	20	24	8	12	16	20	24
Standard	7	19	27	38	68	6	10	20	31	54
IBA 5000	10	22	32	45	81	8	14	26	38	67
Zinc 2500	7	16	32	38	68	6	11	20	34	63
Zinc 5000	9	31	36	47	88	9	13	28	39	64
RHF *	13	42	45	53	90	10	16	29	42	71
Zinc 2500 *	9	29	39	47	80	9	13	28	40	64
Zinc 5000 *	11	35	42	50	82	10	16	33	42	65
IBA 5000 + Zinc 2500	10	34	41	56	87	11	18	31	43	67
IBA 5000 + Zinc 5000	13	43	47	58	105	13	23	32	47	77
IBA 5000 + Zinc 2500 *	11	34	43	54	93	11	21	39	46	79
IBA 5000 + Zinc 5000 *	13	42	56	65	95	14	25	37	50	95
IBA 5000 + O-HBA 20	6	22	33	47	77	10	17	28	33	66
IBA 5000 + p-HBA 10	7	23	33	48	81	11	14	27	33	68
IBA 5000 + Zinc 2500 + O-HBA 20	8	23	34	48	82	10	14	26	28	72
IBA 5000 + Zinc 5000 + O-HBA 20	7	22	30	53	80	11	18	25	34	72
IBA 5000 + Zinc 2500 + p-HBA 10	9	27	31	49	84	10	20	23	36	66
IBA 5000 + Zinc 5000 + p-HBA 10	9	24	37	54	82	11	16	28	36	72
CD (P = 0.05)	1	4	4	4	8	1	2	3	4	6
CV (%)	10	9	7	5	6	8	8	8	7	6

Note : Treatments with an asterisk are in powder formulation; others in solution formulation.

TABLE 4—Effect of treatments on dry weight (mg) of roots per cutting (Means of three replicates)  
Experiment 2

Treatments (ppm)	UPASI - 2					UPASI - 8				
	Weeks after planting					Weeks after planting				
	8	12	16	20	24	8	12	16	20	24
Standard	28	56	71	201	301	15	48	108	135	223
IBA 5000	45	65	92	224	335	20	66	144	150	223
Zinc 2500	12	30	90	148	246	10	41	94	117	232
Zinc 5000	25	51	82	161	249	18	53	135	159	235
RHF*	50	72	147	272	358	22	56	155	182	260
Zinc 2500*	29	65	101	225	260	14	52	134	175	222
Zinc 5000*	38	74	104	227	287	17	75	143	164	233
IBA 5000 + Zinc 2500	46	74	106	246	292	25	81	151	170	248
IBA 5000 + Zinc 5000	54	83	162	292	360	24	82	193	197	258
IBA 5000 + Zinc 2500*	47	80	131	254	317	28	67	160	187	261
IBA 5000 + Zinc 5000*	59	89	159	287	387	32	92	191	200	279
IBA 5000 + O-HBA 20	28	57	105	171	289	20	65	130	147	212
IBA 5000 + p-HBA 10	19	61	119	159	236	22	58	131	146	212
IBA 5000 + Zinc 2500 + O-HBA 20	20	56	99	181	246	20	73	138	158	221
IBA 5000 + Zinc 5000 + O-HBA 20	27	71	90	203	257	24	66	146	165	205
IBA 5000 + Zinc 2500 + p-HBA 10	26	59	121	177	277	21	67	137	145	238
IBA 5000 + Zinc 5000 + p-HBA 10	30	56	89	219	270	24	71	146	157	236
CD (P = 0.05)	3	8	9	22	24	3	6	9	11	15
CV (%)	6	7	7	6	5	9	6	2	4	4

Note : Treatments with an asterisk are in powder formulation, others in solution formulation.

TABLE 5—Effect of treatments on dry weight (mg) of shoot per cutting (Means of three replicates)  
Experiment 2

Treatments (ppm)	UPASI - 2					UPASI - 8				
	Weeks after planting					Weeks after planting				
	8	12	16	20	24	8	12	16	20	24
Standard	8	17	33	92	206	65	81	195	221	389
IBA 5000	14	29	62	117	244	67	92	204	221	406
Zinc 2500	8	13	50	120	224	75	83	170	190	365
Zinc 5000	14	22	57	131	238	88	105	204	240	371
RHF*	16	27	81	138	262	91	123	220	254	437
Zinc 2500*	9	17	56	133	231	74	98	197	245	327
Zinc 5000*	12	20	64	135	243	97	117	204	253	464
IBA 5000 + Zinc 2500	10	20	72	134	264	89	132	210	240	454
IBA 5000 + Zinc 5000	15	21	81	176	291	86	133	223	278	460
IBA 5000 + Zinc 2500*	7	16	71	152	282	85	132	220	258	433
IBA 5000 + Zinc 5000*	15	27	89	182	309	92	155	233	251	473
IBA 5000 + O-HBA 20	8	12	51	106	211	57	78	162	210	431
IBA 5000 + p-HBA 10	8	12	59	111	265	36	72	166	216	325
IBA 5000 + Zinc 2500 + O-HBA 20	11	16	66	110	226	54	66	199	212	365
IBA 5000 + Zinc 5000 + O-HBA 20	7	14	57	108	223	62	98	170	190	375
IBA 5000 + Zinc 2500 + p-HBA 10	8	11	59	128	214	54	89	169	227	401
IBA 5000 + Zinc 5000 + p-HBA 10	7	13	68	115	209	79	91	189	200	374
CD (P = 0.05)	2	3	11	13	17	8	10	12	15	23
CV (%)	12	10	11	6	4	7	2	4	4	4

Note : Treatments with an asterisk are in powder formulation , others in solution formulation.

acid at 20 ppm increased the dry matter accumulation in shoot at the end of 20 and 24 weeks (Fig. 2C). Phenolic compounds by themselves were found to have no effect on rhizogenesis of *Cocoa* sp. (Balasimha and Subramnian, 1983) as also noticed in the present study.

Inhibition of growth by phenolic compounds might be due to the inhibition of metabolism through non-specific metabolic block of ATP synthesis (Khan, 1968). Lee and Skoog (1965) have shown that the response to phenols primarily depends on the promotion or inhibition of IAA oxidation. The depression of root growth due to the application of monophenols noticed in the current investigation may be due to the promotion of IAA oxidase activity leading to reduced auxin levels during rhizogenesis (Krishnamoorthy, 1981 ; Kakkar and Rai, 1986).

In separate combinations with IBA at 5000 ppm, the two phenolic acids improved per cent rooting and other characteristics significantly over the standard treatment, as also, phenolic acids by themselves (Figs 1, 2). However, these mixtures were not superior to IBA alone at 5000 ppm in solution and inferior to RHF in all respects. The negative effect was pronounced in the initial weeks but waned as the experiment progressed.

Balasimha and Subramnian (1983) reported a positive influence of phenolic acids in combination with IBA on *Cocoa* sp. Absence of such an influence on tea cuttings indicates that the response depends on the endogenous levels of phenols; as such, it is likely to be cultivar-/species-/specific. Additionally, an inverse relationship between phenol content and rooting was noticed in coffee ; an optimal ratio between phenol and auxin was suggested by Purushotham, Sulladmath and Ramiah (1984).

Boron at 100 ppm in solution did not have any effect either on rooting or other parameters. Addition of boron to IBA at 5000 ppm did not enhance the efficacy of the IBA ; however, the mixture was superior to boron by itself. Addition of phenolic acids to the mixture of IBA and boron did not improve the efficacy of the mixture ; on the contrary, it depressed dry matter accumulation in roots/shoots in comparison to certain treatments. These findings are in conformity with those of Venkataramani (1962) in tea and Mohandass *et al.* (1982) in geranium.

Boron was omitted in the second experiment because of the lack of response to it in the first.

Zinc at 2500 and 5000 ppm applied as powder formulation was superior to that applied in solution and the standard treatment in the clones UPASI-2 and UPASI-8, with regard to per cent rooting, up to the end of 16 weeks; it was on par with RHF (Table 1). The treatments had no effect on UPASI-17 (Fig. 1B). In both formulations and concentrations, zinc generally, had no effect on the number of roots per cutting (Table 2). Both concentrations of the powder formulation of zinc increased the total length of roots per cutting than that in the standard; both formulations of zinc at 5000 ppm, were found to be comparable to RHF (Table 3).

Dry weight of roots in UPASI-2 and UPASI-8 was either depressed or unaffected towards the end of the experiment by both concentrations of zinc in solution (Table 4); but, the dry weight in UPASI-17 was increased by zinc at 5000 ppm (Fig. 2D). However, zinc applied as powder at both concentrations, significantly increased the dry weight of roots compared to that of the standard, but was inferior to that of RHF.

At the end of the experiment both formulations and concentrations of zinc, enhanced dry weight of shoot in UPASI-2, while only the powder formulation at 5000 ppm was effective on UPASI-8 (Table 5). UPASI-17 responded to zinc at 5000 ppm in solution (Fig. 2C). Zinc at 5000 ppm in powder was comparable to RHF, with respect to shoot weight (Table 5) in the clone UPASI-8.

IBA at 5000 ppm in combination with zinc at 2500 ppm in solution induced higher per cent of rooting only by the end of 12 weeks. It has also enhanced the length and dry weight of roots and shoot weight in comparison to IBA by itself; this combination has also proved to be superior in all respects to zinc at 2500 ppm by itself.

Raising the dosage of zinc to 5000 ppm has significantly enhanced the efficacy of the mixture, inducing superior response over that under IBA alone and zinc by itself at 5000 ppm in solution, in all the parameters (Tables 1-5).

IBA at 5000 ppm in combination with zinc at 2500 ppm in powder formulation proved superior to the mixture in solution, only in enhancing per cent rooting in UPASI-2 and UPASI-8 by the end of 16 and 20 weeks (Table 1), the number of roots in UPASI-2 and total length of roots in UPASI-8 (Tables 2 and 3). The powder formulation of the mixture was not superior to RHF, but, was superior to zinc by itself at 2500 ppm.

Increasing the zinc content of the mixture in the powder formulation to 5000 ppm enhanced its efficacy, inducing higher percentage of rooting by the end of 8 and 16 weeks and achieved significant response in all other parameters. It was also superior to RHF and zinc by itself at 5000 ppm in powder, with respect to all the growth parameters.

The mixture of IBA and zinc at 5000 ppm each, in powder formulation was found to be superior to the mixture in solution inducing significant responses with regard to certain important growth parameters in the three clones used in the study (Figs 1, 2; Tables 1-5).

Addition of either of the phenolic acids at different rates did not alter the efficacy of the mixture containing IBA at 5000 and zinc at 2500 ppm, but, adversely affected the efficacy of the mixture containing IBA and zinc at 5000 ppm each (Tables 1-5).

Zinc, applied by itself or in combination with IBA, generally increased dry matter accumulation, particularly in roots, in respect to the comparable treatments. This may be due to the role of zinc in the synthesis of tryptophane (Audus, 1953) as also its favourable effect on carbohydrate accumulation (Ma Huiqun Raun Yuchen, 1987).

The mixture of IBA and zinc, at 5000 ppm each, in powder formulation has emerged as the best of all the treatments tested in this investigation. (Fig. 3). This formulation has proved to be superior, in all growth parameters, to the rooting hormonal formulation (RHF) which is based only on IBA at 5000 ppm. There is, thus, a distinct possibility to improve the efficacy of RHF that is now in use, by incorporating zinc at 5000 ppm into the formulation.



Fig. 3—Rooted plants under treatments 1. Standard, 2. RHF, 3. IBA 5000 ppm + zinc 5000 ppm (powder).

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