STUDIES ON ORCHIDS OF NORTH-EASTERN INDIA : INFLUENCE OF ECO-PHYSIOLOGICAL FACTORS ON ASYMBIOTIC AND SYMBIOTIC GERMINATION

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CERTIFICATE

We certify that the thesis entitled **Studies on orchids of north-eastern India : Influence of eco-physiological factors on asymbiotic and symbiotic germination** submitted by Mr. **Avadh Naresh Raghuwanshi,** for the **Degree of Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under our supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the **Ph.D. Degree.** This work has not been submitted for any Degree of any other University.

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Place : Shillong
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CONTENTS

INTRODUCTION.

Orchids are no doubt the most beautiful and wondrous among all the flowering plants in the world. These extraordinary plants are economically important primarily due to their horticultural and floricultural appeal. Orchids as plants of ornamentation were known to ancient India. The flowers of Aerides and Rhynchostylis were used to be associated with the festivals and adorned by the ladies as "Draupti pushapa" and "Sheta pushapa" (Hegde, 1985). Some of the orchids were also used in "Ayurvedic" system of medicines, and also as food. Orchids, taxonomically represent the most highly evolved family among monocotyledons. They comprise of 800 genera and 35,000 species, distributed throughout the world world (Bose and Bhattacharjee, 1980). Perusal of literature reveals the existence of 925 Indian orchid species (Jain and Mehrotra, 1984). Out of 925 species, 284 are categorised as endemic, 30 have become extinct and 105 are endangered (Hegde, 1985). Six hundred orchid species are reported from the North-Eastern India, which represent about 65% of Indian and 1.7% of world orchid population.

Orchids are distributed in various forests, grasslands and agro-ecosystems. The North-Eastern region of India is rich in tropical wet evergreen, semi-evergreen, moist deciduous, subtropical broad leaved and alpine forest types, which provides conducive environment for the establishment and growth of epiphytic and terrestrial orchids due to varied climate, soil, vegetation and topography.

The North-East India is mainly inhabited by tribals, who commonly practise 'Jhum' cultivation. Besides, the increased population pressure coupled with other human activities, i.e. deforestation, construction of roads, dams, extraction of wood for fuel and furniture, have fastened the process of orchid depletion at a higher rate from their natural habitats.

Orchids are perennial with epiphytic or saprophytic habit in nature. The vegetative parts show great variations. The majority of flowers of orchids are not pollinated, their ovules are not fertilized and capsules are rarely formed. But where there is pollination a large number of seeds are produced which may range from 5000-1,00,000 per capsule. However, their germination is very poor (3-5%) under natural conditions, because of the particular fungal requirement.

Role of mycorrhizal fungi in orchids is emphasised in converting the complex reserve food in seed-coat into simpler forms (Harvais and Hadley, 1967b) which is made available to the germinating seeds and it helps in early development of seedlings (Arditti, 1967, 1979). In nature the growth of protocorms is arrested as long as infection by suitable mycobiont is achieved (Meyer, 1966). Efficiency of fungi in hydrolysing and mobilising the starch and other complex carbohydrates has been shown by some workers (Purves and

Hadley, 1975). The enhanced growth at early stage of seedling development is also assigned to its increased enzymatic activity in root region due to mycobiont which may also provide vitamins and other growth factors (Harvais and Pekkala, 1975).

Mycorrhizal fungi might produce some growth hormones like auxins, cytokinins, etc.. It was postulated that such growth regulators might not only facilitate the infection of cells, but may also stimulate cell elongation, mobilization of sugars and even cell division (Hayes, 1969). Various media have been used for symbiotic germination and seedling growth of orchids. Clements (1979) found Oat medium most suitable for symbiotic seed germination. Linden (1980) studied a number of media for aseptic germination of seeds of orchids and found Burgeff's and Fast's media to support good germination and growth. The asymbiotic germination of seeds can take place if the medium is exogenously supplied with growth hormones, vitamins and trace elements (Harvais, 1982; Nakamura, 1982).

Harvais and Hadley (1967a) studied the symbiotic relationship between fungal symbiont and orchid hosts. Further, these authors (1967b) observed that the seedling development in Orchis purpurella was better in symbiotic cultures under low light and low temperature conditions than

in asymbiotic conditions.

A major benefit of fungus root association is the enhanced absorption of nutrients by the roots from non-fertile or moderately fertile soils. Absorption of phosphorus appears to be particularly enhanced by the mycorrhizal system. The fungal-symbionts capacity to produce acid phosphatase appears to be an important part of mycorrhizal absorption of phosphorus (Bowen, 1973). Gianinazzi (1978) has reported that mycorrhizal specific phosphatase activity is closely linked with the development of both mycorrhizal infections and the infected host plant. Infection of roots by mycorrhizal fungi may also be influenced by phosphatase concentration.

Orchid population is mainly influenced by the factors like temperature, light, p.I of the soil and bark, amount of rainfall, humidity, type of soil and vegetation, etc. in the natural conditions (Hegde, 1985).

Most of the studies on orchids are related to the effect of growth hormones, vitamins, nitrogen sources, and complex additives (Knudson, 1922; Quednow, 1930; Meyer, 1943; Noggle and Wynd, 1943; Mariat, 1952; Withner, 1959a; Fonnesbech, 1972; Mead and Bulard, 1975). However, work on the effect of some environmental factors, i.e., light, substrate pH, and temperature on seed germination of orchids, is meagre (Ueda and Torikata, 1972). And the role of mycorrhizal

fungi in phosphorus uptake and phosphatase metabolism in orchids has not been worked out in detail. Similarly, not much investigations have been done on the growth hormones production by mycorrhizal fungi of orchids. However, some information is available on the growth regulators production by some ectomycorrhizal fungi (Ulrich, 1960; Crafts and Miller, 1974; Ng et al., 1982) and by vesicular arbuscular mycorrhizal fungi (Barea and Aguilar, 1982).

Therefore, the following work was undertaken to study the effect of some eco-physiological factors on in vitro cylture of orchids both in asymbiotic and symbiotic conditions and some aspects of their metabolism. The present investigations have been categorized as follows:

i. Studies on the effect of temperature, pH and light on asymbiotic seed germination and subsequent seedling growth of orchids.

ii. Studies on the effect of temperature, pH and light on symbiotic seed germination and subsequent seedling growth of orchids.

iii. Growth hormones production by mycorrhizal fungi of orchids.

iv. Phosphorus uptake and phosphatase activity in mycorrhizal seedlings of orchids.

REVIEW OF LITERATURE.

Orchidaceae, is one of the largest and most diverse of all plant families (Garay, 1960). Its representatives may be found from the Arctic to the Antartic in bogs, deserts, valleys, plains, hills, mountains and even belowground (Hatch, 1953). Size of orchid plants varies from 3-4 mm to several meters, whereas flowers may range from 2-3 mm to 15-20 cm or more in diameter. Such wide variations necessitate numerous adaptive characteristics; for example certain orchids may contain little or no chlorophyll (Senn, 1927) and are therefore, saprophytic and parasitic (Burgeff, 1932; Hamada, 1939; Campbell, 1962, 1963; Hamada and Nakamura, 1963). The specialized flower structure has resulted in characteristic pollination mechanisms (Darwin, 1888) which include pseudo-copulation (Ames, 1948), self-pollination (Knudson, 1956) and cross pollination (Dunsterville and Garay, 1959). The most interesting adaptive features of the orchidaceae are those occurring in the physiology of their seed germination (Constantin, 1917).

At least three distinct periods may be delineated in the investigation dealing with the orchid seed germination. During the initial period, investigations were limited to the study of symbiotic relationship in orchids (Bernard, 1899, 1900, 1909; Ramsbottom, 1922a,b, 1929; Burgeff, 1936, 1959). In the early phases of the second period, which began with Knudson's publications, the merits of symbiosis and asymbiosis

were argued (Bultel, 1924, 1925; Constantin, 1925, 1926; Knudson, 1924, 1930; Burgeff, 1936, 1959). Later on, the effects of various ions (La Garde, 1929; Wynd, 1953), sugars (Quednow, 1930; Wynd, 1933b), vitamins (Meyer, 1943; Noggle and Wynd, 1943; Bahme, 1949), hormones (Mariat, 1952; Withner, 1959a) and other complex organic additives (Knudson, 1922; Quednow, 1930) were examined.

Reissek (1847) first observed fungal association with roots in orchidaceae. Frank, afterwards coined the term "mycorrhiza" in 1885, to symbiotic association of fungi and roots. Wahrlich (1886) reported that the mycorrhizal association is a wide spread phenomenon in most of the orchid species. Stahl (1900) described the mode of symbiosis with orchids and suggested the term "holomycotrophy" for the association (nourishment entirely through digestion of fungi; holes: total, mykes: fungus; trophe: nourishment).

First isolation of fungal endophyte from the germinated seeds and roots of orchids was done by Bernard (1903, 1904). The endophytes isolated from the Cattleya sp., Epipactis sp. and Cypripedium sp. were tested for their infection in the seeds of Odentoglossum sp.. He observed the lethal effect of fungi on the germinating seeds and thus concluded the association as parasitic instead of symbiotic. Bernard later on (1909) noticed that most of the orchid seeds

depended upon the mycorrhizal fungi for the germination and early growth under natural conditions, but they showed host specificity. He further (1911) observed the defense reaction of host to the invasion of fungal endophyte.

Burgeff (1909) observed fungal coil formation within the host cells. The coils contained protein, glycogen and fat. Host cells digest these coils through the 'tolypophagy' type of digestion. Based on the mode of fungal digestion, he (1959) categorised the digestion in orchids into three types, i.e., tolypophagy, ptyophagy, and themiscophagy.

Kusano (1911) reported that heterotrophic orchid (Gastrodia eleta) may form symbiotic association with Armillaria millea. He also noticed that the bulb remained quiescent until the fungus attacked. Duggar (1915) gave detailed account of the genus Rhizoctonia. Duggar and Davis (1916) demonstrated nitrogen utilization of Phoma radicis, a mycorrhizal fungus with some orchids. The view that orchid seeds depend upon fungal association for germination was supported by Romsbottom (1922a). He again (1922b, 1927) advocated the specificity of the fungus in relation to the orchids. Norbacourt (1923) found that the growth of the fungus is inhibited by the antifungal substances present in the tubers of certain orchids.

In contrast to reports on fungal requirement of orchid seed germination, Knudson (1925) suggested that fungus is not necessarily required for seed germination, if the synthetic medium is supplemented with various substances like vitamins, amino acids, and growth hormones.

Rayner (1926) observed the presence of fungus in the seedlings of Goodyera procera. He opined that the association is a beneficial phenomenon. He (1929) further noticed that mycorrhiza of Neottia nidus-avis can fix the atmospheric nitrogen. While studying the germination of orchid seeds, Curtis (1936, 1937) noticed that some fungi were pathogenic. He (1939) also reported non-host specificity in case of some of the fungi. Downie (1943) isolated two strains of Rhizoctonia from the germinating seeds, and pointed out that soil can influence the distribution of endophytes in terrestrial orchids. She (1957) observed that Rhizoctonia constantly formed symbiotic association with orchids. Further, she reported (1959a,b) that fungal endophytes are capable of enhancing germination and provide some specific compounds like vitamins, etc. to the germinating seeds.

Gaumann and Jaag (1945) isolated the endophytes from orchids and studied the defense reaction of Orchis militaris to the fungi using tissue culture techniques and observed anti-fungal substances in the host cell.

Slankis (1948) reported the production of auxins by the mycorrhizal fungus Boletus variegatus, but no identification of the auxins was made. Studies employing modern methods to characterize specific compounds have demonstrated the production of auxin by other fungi and bacteria. Diplodia, a member of the fungi imperfecti grew uniformly on synthetic media and synthesized indoleacetic acid (IAA) both in the presence and absence of tryptophan (Gentile and Klein, 1955).

Ulrich (1960) screened many mycorrhizal fungi for the production of auxins, in the liquid culture medium and in the sporophores. He observed the production of indole compounds, i.e., IAA, indolebutyric acid (IBA), indoleglycolic acid (IGA), indolelactic acid (ILA) and indole pyruvic acid (IPyA) in most of the mycorrhizal fungi.

Gauman et al. (1960) studied the defense reaction of Orchis militaris to the fungi, using tissue culture techniques and observed the anti-fungal substances in the host cell. They (1960) also isolated the active substance and identified it as 'Orchinol'. Gauman and Hohl (1960) confirmed the production of orchinol in the living cells of orchid tubers in vitro.

Hadley and Perombelon (1963) observed the production of pectic enzymes by the Rhizoctonia solani, R. repens and R. goodyera repentis in vitro. Harvais (1965) studied some

aspects of the symbiosis of Orchis purpurella.

Harvais and Hadley (1967a) suggested the symbiotic relationship between fungal symbiont and host, particularly in orchid mycorrhiza. They (1967b) observed that the seedling development in O. purpurella was better in symbiotic cultures under low light and low temperature conditions than in asymbiotic conditions. Zeigler et al.,(1967) studied the influence of various media and photoperiods on growth and aminoacid contents of orchid seedlings.

Gogala (1967, 1971) demonstrated that the ectomycorrhizal forming fungus Boletus elutis var. pinicolus synthesized several growth hormones. He observed that the fruiting bodies and the mycelia of this fungus in pure culture, as well as the culture medium in which the mycelia had grown for one month, contained three indole derivatives, corrosponding to IAA, and a compound which seemed to be tryptophan and also growth hormones related to gibberellins and cytokinins.

Hadley and Harvais (1968) studied asymbiotic growth promotion in orchid-protocorms by certain growth hormones. Hadley (1969) found that cellulose promoted seed germination when supplied alongwith glucose both asymbiotically and symbiotically. He (1970) further noticed the non-aggressive nature of some endophytes and categorised them as

'Ubiquitous' endophytes. Hadley and Williamson (1971) found that symbiotically infected protocorms of Dactylorhiza purpurella showed linear increase in their length and width in contrast to non-infected ones. They (1972) also studied the influence of mycorrhizal infection and its intensity on the structure of nucleus of the host cells. Nuclear hypotrophy was observed in the host cells adjacent to the infected tissue.

Goh (1971) studied the effect of pH on the absorption of phosphate by the terrestrial roots of two orchid hybrids. He observed most efficient uptake in the range of pH 5.0 to 5.5 in Vanda, whereas in Arachnis pH 5.5 appeared to be the optimum. In both the cases, they showed the preferences for slightly acidic pH and uptake was much slower at alkaline pH. Ueda and Torikata (1972) investigated the effect of light and culture medium on adventitious root formation by Cymbidiums in aseptic culture and found no root formation in the shoots cultured in the dark, whereas white and blue light induced good root formation.

Smith et al. (1973) studied the uptake of glucose, trehalose and mannitol by the leaf slices of the orchid Bletilla hyacinthina, and found that the absorption of all three carbohydrates was reduced by low temperature.

Hijner and Arditti (1973) reported the production of vitamins by the orchid mycorrhizal fungi in the symbiotic conditions. The production of cytokinin: transzeatin and transribosyl zeatin in the culture medium of mycorrhizal fungus Suillus punetipus was reported by Crafts and Miller (1974). Similarly, they also found sufficient quantity of cytokinins in the culture medium of Rhizopogon ochraccorubens.

Hadley and Purves (1974) showed the movement of carbon^{14} from host to the fungus. Hadley (1984) explained the uptake of ^{14}C glucose by asymbiotic and symbiotic protocorms of Goodyera repens.

Harvais and Pekkala (1975) found that the fungus could produce vitamins: nicotinamide, and thiamin in yeast extract supplemented liquid medium. The plantlets of Orchis purpurella in asymbiotic and symbiotic conditions were raised by Purves and Hadley (1975). They further (1976) suggested that the protocorms of Goodyera repens develop faster if infected with mycorrhizal fungi than the non-infected ones. Peschke and Volz (1978) observed the association of Fusarium moniliforme with various orchid species.

Clements and Ellyard (1979) studied symbiotic germination in the terrestrial orchids of Australia. They found stimulatory effect by most of the endophytes prior to seedling development. Vij and Datta (1981) investigated the

distribution of fungi in the roots of Herminium angustifolium (Benth). They isolated the fungal symbionts resembling with Rhizoctonia sclerotina (Burgeff). Vij and Sharma (1983) carried on a survey of mycorrhizal association in some terrestrial and epiphytic orchids of northern India.

Dexheimer and Serrigny (1983) studied the ultrastructure of endophyte of Epidendrum ibaguense and found more accumulation of alkaline and acidic phosphatase in infected cells.

Alexander and Hadley (1983) isolated endophyte from Goodyera repens and identified it as Rhizoctonia goodyera repentis. The symbiotic efficiency of the fungi with the germinating seeds of G. repens was also tested. The effect of fungicide on the mycorrhizal infection in G. repens (Alexander and Hadley, 1984a) and phosphate uptake (Alexander and Hadley, 1984b) have been studied. They (1985) also studied the movement of carbon between host and mycorrhizal endophytes at the time of G. repens development in the symbiotic conditions. Lin and Molnar (1983) observed the effect of photoperiod and high light intensity on the flowering of orchids.

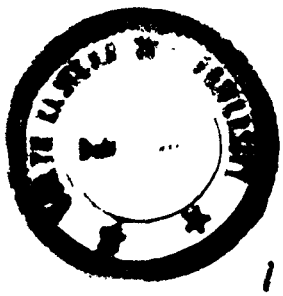
Hills et al. (1984) studied the effect of different chemical compounds on the seedling development of Cattleya aurantiaca. Filipello et al. (1985) isolated some

endophytes from the orchids of Italy and carried morphological, cytological, and cytochemical studies. Williams (1985) observed that Rhizoctonia strains form association with orchids from the pot cultures of vesicular arbuscular mycorrhizal fungi.

Sharma and Tandon (1986) studied the influence of growth regulators on asymbiotic germination and early seedling development of Coelogyne punctulata Lindl.

Katiyar et al. (1986) reported the effect of organic supplements on the seedling growth of an endangered orchid species Coelogyne punctulata.

Raghuwanshi et al. (1983) reported the asymbiotic seed germination in epiphytic orchids. These authors further reported (1985) the effect of synthetic media on asymbiotic seed germination and seedling growth of Dendrobium nobile and Sarcanthus pallidus, and found better germination and growth on Burgeff's and modified Kn C medium. Subsequently, they (in press) studied the effect of temperature on asymbiotic seed germination and seedling growth of orchids, and noticed better results between the temperature range of 20-30°C. Further, a pH range from 4.0 to 6.0 was found better for the seed germination and seedling growth (Raghuwanshi et al., 1986; in press).



CLIMATE AND VEGETATION OF COLLECTION SITE.

Survey of orchids was carried out from various grasslands, agricultural lands and forest of Khasi Hills, Garo Hills and adjacent areas of Meghalaya (Fig. 1). Plants of different epiphytic and terrestrial orchid species were collected and grown in charcoal and soil mixture, filled in earthen pots. Some epiphytic orchids were grown on old dead logs under semi-controlled conditions (in net houses) in Botany Department, School of Life Sciences, North-Eastern Hill University, Shillong (latitude, 25.34°N, longitude 91.56°E, altitude 1956 mm).

Soil

Shillong plateau has mild undulatous topography. The soil is laterite with reddish brown colour and the texture is sandy-loam at the surface end and silty-loam at deeper layers. It has originated from the hard rock representing gneses, schists and granites. Zimba (1977) has proposed that Shillong plateau and its surrounding hills might have uplifted from sea bed alongwith origin of great Himalaya during mesozoic and early tertiary times. The soil is rich in organic matters and nitrogen but acidic in reaction.

Vegetation

The vegetation of Meghalaya can broadly be

Fig. 1 : Map of Meghalaya showing the places of orchid collection. (● = places of collection; ⊗ = study site).

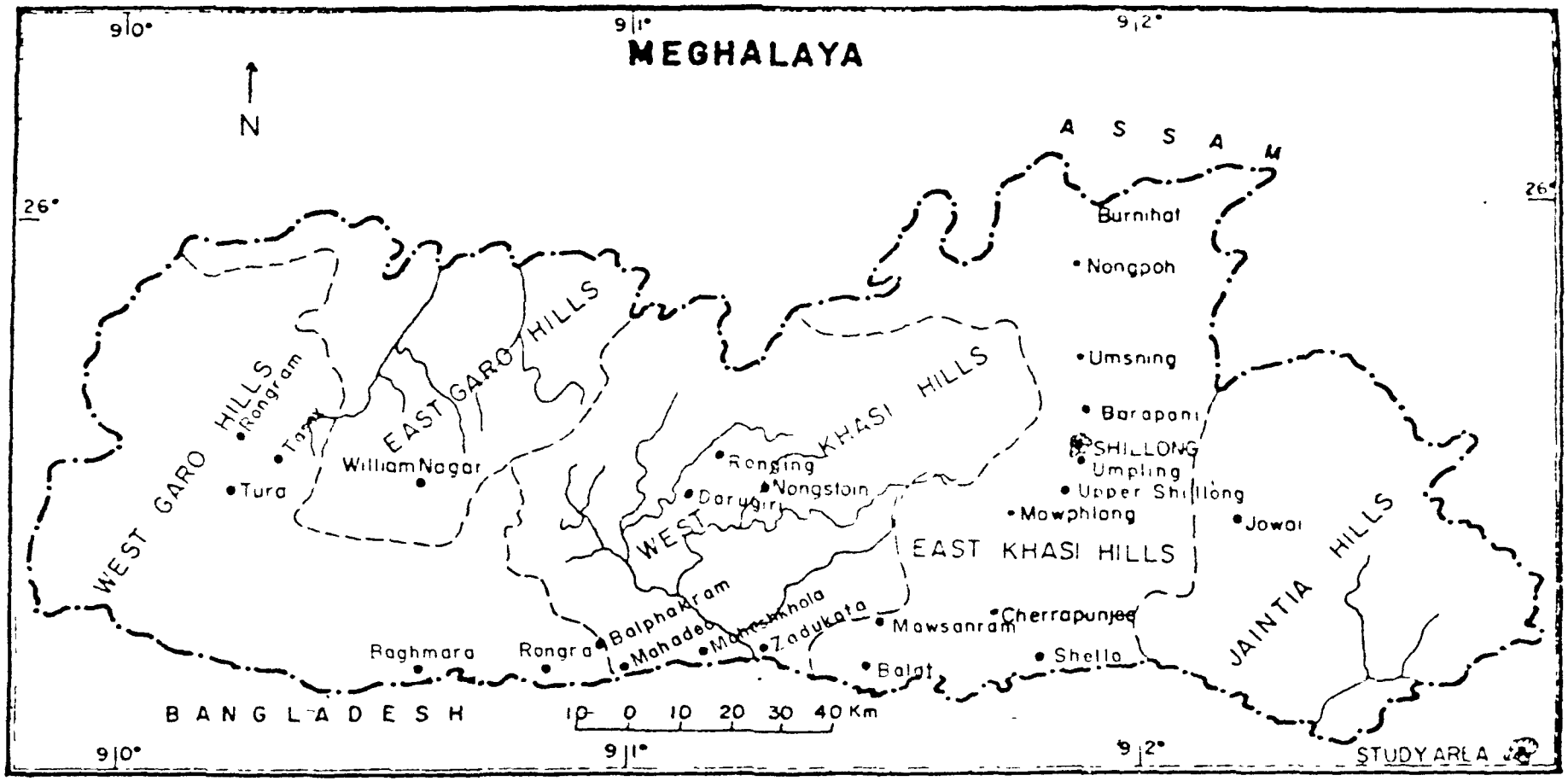

MEGHALAYA
N
9|0°
9|1°
9|2°
26°
ASSAM
Burnihat
Nongpoh
Umsning
Barapani
SHILLONG
Umpling
Upper Shillong
Mawphlang
Jowai
Cherrapunjee
Mawsanram
Shella
Balat
Zadukata
Mahadeo
Balpakram
Rongra
Raghmara
Tura
Rongram
William Nagar
Ranging
Nongstoin
Darugiri
WEST GARO HILLS
EAST GARO HILLS
WEST KHASI HILLS
EAST KHASI HILLS
JAINTIA HILLS
BANGLADESH
10 0 10 20 30 40 Km
STUDY AREA

Fig. 1

classified into:

1) Subtropical forest
2) The mixed evergreen forest
3) Temperate forest
4) The rolling grassland.

The sub-tropical pine forests represent biotic climax, dominated by Pinus kesiya alongwith some tree species like Alnus nepalensis, Schima spp., Quercus spp., Cedrus deodara, Cryptomaria japonica, etc. and some other trees, shrubs and herbaceous species like Sympiocus spp., Rhododendron arboreum, Lantana camara, Eupatorium spp., Anaphilis spp. and Desmodium spp. etc. Mixed substropical forests are confined to restricted areas and are much disturbed. These are dominated by Schima spp., Quercus spp., A. nepalensis, Erythrina arborescens and a number of rosaceae members.

Temperate forest are found from 1800 m and above. The true temperate vegetation represents the richest flora (in preserved/sacred forests) and gives an indication that probably the entire area was once covered by such type of dense vegetation but now has been disturbed due to human activities. In such forests the common trees namely Q. griffthii, Myrica esculenta, Betula alnoidus, R. arboreum, Castanopsis spp., Photenia notoniam etc., shrubs like, Daphne spp., Osbeckia spp. and beautiful orchids like

Dendrobium spp., Coelogyne spp., Cymbidium spp., Oberonia spp., Pleione spp., Pholidata spp., Eria spp., etc. are commonly encountered. The epiphytic plant species comprise of lichens, mosses, and ferns as dominant species on the tree trunks and branches of old trees in moist humid forests.

The grasslands represent most important vegetation type (ground flora) of this region. Most of the grasslands are in the different stages of sereal succession. The primitive agricultural practice, i.e., shifting cultivation locally known as 'jhumming' is commonly used for raising the agricultural crops in the North-Eastern region. The extensive use of 'jhumming' is main factor in resulting the genesis of grasslands in the region. Other biotic and human disturbances have also promoted the process. The grasslands consist of dominant species like Paspalum dilatatum, Pennisetum cladestinum, Imperata cylindrica, Penicum brevifolium, Cyperus, spp., Fimbristylis spp., Arundinella spp., Trifolium repens, Cassia spp., Desmodium spp., etc. and some beautiful terrestrial orchids like Spathoglottis pubescens, Spirenthus spp., Herminium spp., Habenaria spp., Arundiana spp., Paphiopedilum spp. etc.

Climate

Shillong climate is very much influenced by the

Fig. 2 : Meteorological data for minimum and maximum temperatures (°C), relative humidity (%) and rainfall (mm) for the year 1982-83 of the study site in Shillong.

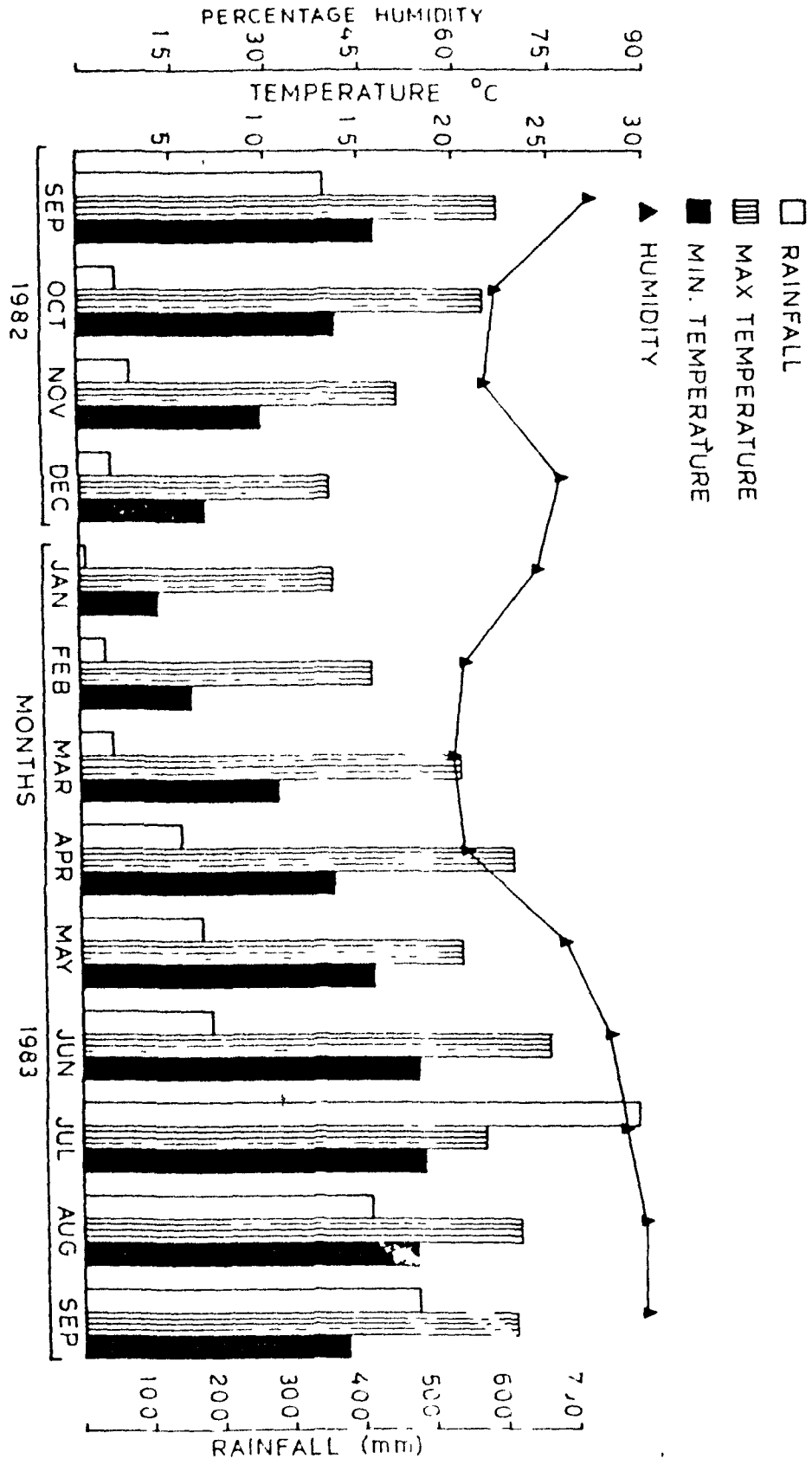

PERCENTAGE HUMIDITY
15
30
45
60
75
90
TEMPERATURE °C
5
10
15
20
25
30
RAINFALL
MAX TEMPERATURE
MIN. TEMPERATURE
HUMIDITY
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
1982
1983
MONTHS
100
200
300
400
500
600
700
RAINFALL (mm)

Fig. 2

south-west monsoon and north-eastern winds.

Therefore, the year can be divided into four seasons:

i) Spring season - March and April

ii) Summer (rainy) season - May to September

iii) Autumn season - October and November

iv) Winter season - December to February.

During spring season atmosphere gets warm gradually as compared to the preceeding winter months. The maximum temperature reaches during the period of April to June-July. The average maximum temperature at Shillong recorded during the study period was 24.8°C and the average minimum temperature 12.5°C. Rain starts at the end of April and continues upto September. The monthly average rainfall normally is 212.02 mm. The average humidity (%) ranges between 61 to 88.5 (Fig. 2). October and November represent a typical autumn season with mild cold and usually with less rain. The winter season can be characterised by low temperature, from cool to cold one. The temperature drops down to a minimum of 1°C in the early period of January and occasional frost can appear. Sometimes there is rain during the month of March which is helpful for the germination and development of most of the trees, plants, and herbs.

Plate 1 : Cymbidium giganteum (Fig. A) and
Cymbidium elegans (Fig. B)
showing epiphytic growth in
nature.

PLATE 1

A

B

Plate 2 : *Thunia alba* showing epiphytic growth in nature (Figs. A and B)

PLATE 2

A

B

Plate 3 : Seedlings (150 day-old) of *Cymbidium giganteum* (Fig. A) and *C. elegans* (Fig. B), grown in Knudson 'C' Medium.

PLATE 3

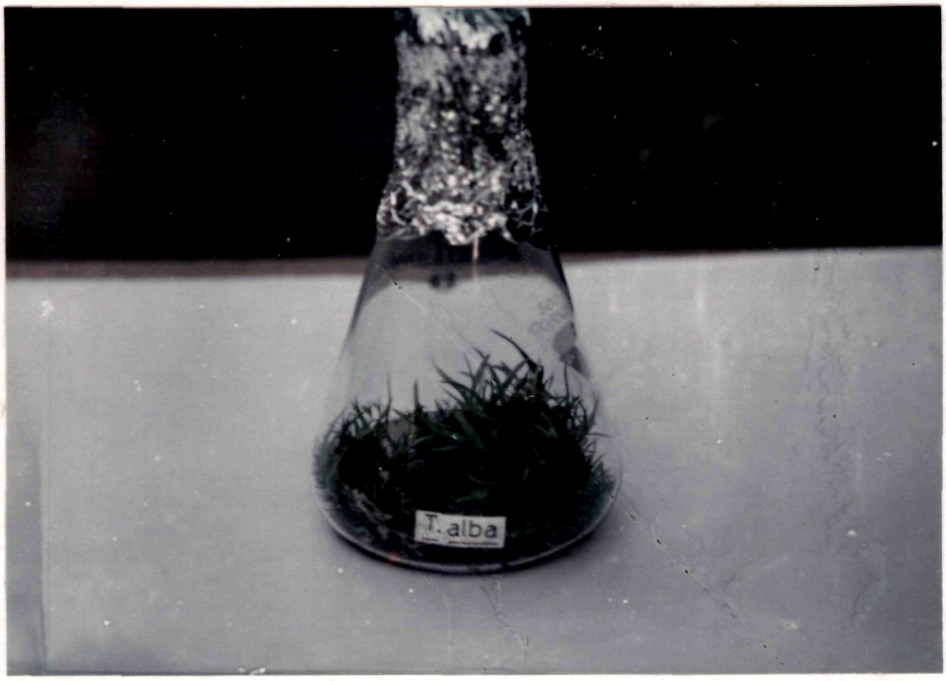
A

B

Plate 4 : Seedlings (150 day-old) of *Thunia alba* (Figs. A and B), grown in Knudson 'C' Medium.

PLATE 4

A



B

MATERIALS AND METHODS.

The orchids collected from forests were grown in charcoal and soil mixture, filled in earthen pots and were kept in net houses of the Botany Department of the North-Eastern Hill University, Shillong. Cymbidium giganteum, C. elegans and Thunia alba, were selected for the present study on the criteria that they are threatened species, have attractive flowers and show fast germination of seeds.

I. Asymbiotic seed germination and seedling growth

The unopened green capsules (approximately 3-5 months old) of Cymbidium elegans, C. giganteum and Thunia alba were collected and surface sterilized by dipping them in 5% (W/V) sodium hypochlorite solution for 10 minutes and then rinsing in absolute ethanol for a few seconds. The sterilized capsules were washed 2 to 3 times in sterilized distilled water and were cut into two half pieces with sterilized scalpel and the seeds were taken out with the sterilized needle. These seeds were then transferred to the slopes on modified Knudson 'C' medium (Bose and Bhattacharjee 1980). All the inoculation processes were carried out under aseptic conditions using laminar flow chamber. The pH of the medium was adjusted to 5.0 by adding 1 N HCl and 1 N NaOH solution before autoclaving. Twenty five and 50 ml of sterilized medium was poured in culture tubes and conical flasks (25 x 150 mm and 100 ml), respectively and allowed to

solidify. Twenty five replicates of each species were incubated in B.O.D. incubators in dark conditions for two months and thereafter, under fluorescent light condition (1300 lux; 12 hr photoperiod) upto five months. Cultures were observed after 60 days and subsequently at 30 days intervals upto 150 days. Percentage of seed germination, average area of seedlings/plantlets, average number and area of leaf primordia/leaves, average number and area of rhizoids/roots and growth index were the parameters used to study germination and subsequent seedling growth. More than 100 seeds/protocorms were observed to calculate percentage of seed germination and seedling growth. The quantify normal growth of seedlings, seedling/plantlets were divided into six different developmental stages (Spoerl' 1948; Mariat, 1952; Arditti, 1967b). Percentage of each developmental stage was multiplied by the developmental stage number and all sums so obtained were added. The total obtained was the growth index. Average area of seedlings/plantlets was measured by calculating average length and breadth of all the growth stages present and multiplied. Similarly, average number and area of rhizoids/roots and leaf primordia/leaves was also obtained for the different growth stages, during seed germination and seedling development of orchids. The effect of following treatments on seed germination and seedling growth of orchids was studied.

1) Temperature treatments

The seeds were incubated at different temperatures, viz., 20°C, 25°C, 30°C and 35°C as described above.

2) pH treatment

The seeds were sown in modified Knudson 'C' medium having different pH, viz., 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0 adjusted by adding either 1 N HCl or 1 N NaOH before autoclaving it. The cultures were incubated at 25° ± 2°C under 12 hr photoperiod of 1300 lux.

Composition of modified Knudson 'C' medium is given below:

Calcium nitrate 0.20 gm/l; monobasic potassium phosphate 0.15 gm/l; magnesium sulphate 0.25 gm/l, ammonium sulphate 0.10 gm/l; potassium nitrate 0.18 gm/l; disodium EDTA 74.6 mg/l; ferrous sulphate 25.0 mg/l; boric acid 6.2 mg/l; maganese sulphate 0.75 mg/l; zink chloride 3.9 mg/l; potassium iodide 0.80 mg/l; sodium molybdate 0.25 mg/l; copper sulphate 0.025 mg/l; cobalt chloride 0.025 mg/l; thiamin hydrochloride 0.30 mg/l; pyrodoxine hydrochloride 0.30 mg/l; riboflavin 0.30 mg/l; sucrose 20 gm/l; and agar 10 gm/l.

3) Light treatment

a) Light intensity

The cultures were incubated for 150 days,

under different light intensities, i.e., 1500, 3000 and 5000 lux, and in the dark conditions. Various light intensities were obtained by 40-Watt white fluorescent tubes.

b) Light quality

Light of different colours, viz., red, blue, green and white were provided by wrapping cellophane paper around the tube lights, in the B.O.D. incubators. One set of culture tubes was also kept under dark conditions.

c) Photoperiod

The seeds were sown on Knudson 'C' medium and were incubated at room temperature (20-25°C) under fluorescent light (3500-4000 lux). Photoperiods of 8, 12, 16, 20 and 24 hr were provided with the help of timers, using 40 watt fluorescent tubes.

II. Symbiotic seed germination and seedling growth

1) Isolation and culture of endophyte

The infected roots of the orchids were selected and cut, washed with tap water and surface sterilized with sodium hypochlorite solution (5%) for 10-15 minutes. The sterilized roots were washed several times in sterilized distilled water. Root sections of 1-2 mm thick were cut using sterile scalpel. Two media, i.e., Malt Extract Agar (MEA) (Booth, 1971) and Potato Dextrose Agar (PDA) (Hadley and Ong, 1978)

were used for the isolation of fungal endophyte from the roots. Chemical composition of the media is given below:

a) Malt Extract Agar

Malt Extract	- 20 g
Agar	- 20 g
Distilled water	- 1000 ml.

b) Potato Dextrose Agar

Potato Extract	- 200 g
Dextrose	- 15 g
Agar	- 20 g
Distilled water	- 1000 ml.

Each nutrient medium was sterilized at 15 p.s.i. for 15 min. and poured into pre-sterilized petriplates. Sterilized root sections were teased and inoculated in centre of petriplate on the nutrient medium; one section was used for each petriplate. Twenty petriplates were used to isolate the fungi for each orchid species. The inoculated plates were incubated at 25 $\pm$ 2°C in a B.O.D. incubator. After one week, white mycelium appeared around the inoculated root sections. The fungus was retransferred onto freshly prepared PDA medium and reincubated at 25 $\pm$ 2°C in a B.O.D. incubator for one week. These fungi were sub-cultured on MEA medium in culture tubes for stock culture and kept at 4°C in the refrigerator. All the fungal isolates were grown on similar medium and those

varied in their morphology were considered as separate strains of Rhizoctonia. These fungi were represented by the code number RH and RA - (Rhizoctonia). Only those fungi were considered for symbiotic seed germination and seedling growth studies, which formed the symbiotic relationship with orchids. Fungal isolate number and their sources are mentioned below:

Isolate	Orchid source of the fungus
RA 4	Ione candida (Lindl.)
RA 5	I. candida (Lindl.)
RA 20	Cymbidium giganteum (Wall.)
RA 40	Coelogyne punctulata (Lindl.)
RH 15	Dendrobium longicornu (Lindl.)
RH 36	Pleione maculata (Lindl.)
RH 46	Coelogyne ocultata (Hook.)
RH 51	C. prolifera (Lindl.)
RH 54	Liparis distans (Clarke.)
RH 61	Thunia alba (Reichb. f.)

2) Preparation of cultures

The stock cultures of fungal isolates were maintained on PDA slopes at 4°C. Five isolates, viz., RA 20, RA 40, RH 15, RH 36 and RH 54 were selected and transferred to the fresh medium in the petriplates. The plates were incubated at 25 ± 1°C for 7 days.

Oat medium (Clements, 1979) was used to raise symbiotic seedlings *in vitro*. The nutrient medium contained 3.5 g powdered oats; 0.1 g yeast extract; and 10.0 g Agar, per litre. pH of the medium was adjusted to 5.0 by using digital pH meter. Medium was autoclaved for 15 minutes at 15 p.s.i. and poured in pre-sterilized culture tubes (25 x 150 mm). Seeds from the surface sterilized capsules of *Cymbidium elegans*, *C. giganteum* and *Thunia alba* species were sown on the agar slopes of oat medium, under aseptic conditions.

Different mycorrhizal fungi were also inoculated along with the seeds of each species separately at the time of sowing. Twenty replicates were made for each orchid species. The uninoculated (asymbiotic) and inoculated cultures were maintained under several combinations of illuminations, temperatures and pH, to investigate their influence on symbiotic culture of orchids *in vitro*.

3) Incubation of cultures

Fungal inoculated and control cultures were incubated under different culture condition such as temperature (20°C, 25°C, 30°C and 35°C), light (photoperiods 8, 12, 16, 20 and 24 hr), light intensities 0, 1500, 3000 and 5000 lux and light qualities (red, green, blue, white and dark) and pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0) of the medium

according to the details described above.

4) Observation of cultures

In general three replicate tubes were taken for each treatment. The seeds and protocorms (germinated seeds) were removed from each tube for microscopic examination and the percentage germination was determined. To study seedling growth, the protocorms/plantlets were divided into six different developmental stages and growth index was determined (Spoerl, 1948). Average area of seedlings/plantlets was observed by multiplying average length and width of all the growth stages occurred at the time of observation. Similarly, average number and area of rhizoids/roots and leaf primordia/ leaves were also determined on the basis of developmental stages. Statistical analyses (standard error and analysis of varience) were carried out following the method of Croxton et al. (1975).

III. Detection and identification of growth hormones in mycorrhizal fungi of orchids

1) Culture of mycorrhizal fungi

Seven mycorrhizal fungi, i.e. RH 15, RH 36, RH 51, RH 46, RH 54, RH 61 and RA 40 were used to detect growth regulating substances in pure culture. The Hagem liquid medium (Fries, 1943) having the following composition per

litre: 5.0 g malt-extract; 5.0 g glucose; 1.0 g tryptophan; 0.5 g KH_2PO4; 0.5 g $MgSO4.7H_2O$; 0.5 g NH4Cl; and 0.5 ml (1%) solution of $FeCl_3$ was prepared and autoclaved at 15 p.s.i. for 15 minutes and 100 ml of the medium was poured in 250 ml capacity conical flasks. Inoculum from pure culture of the fungus was inoculated in the nutrient medium under sterilized conditions. Cultures were incubated at 24 ± 1°C in the dark. After 10 days of growth, cultures were investigated for the presence of hormone-like substances, and further observations were made at 10 days intervals upto 30 days and then after 60 days.

2) Extraction of growth hormones from culture filtrates

The extraction, detection and identification of growtn hormones were carried out following the method of Mahadevan *et al.* (1982) as described below:

(a) Auxins

The nutrient media in which mycorrhizal fungi had grown were centrifuged at 2,000 g in Remi centrifuge for 30 minutes, and the supernatant was acidified with 1N HCl to pH using a digital pH meter and extracted immediately with three aliquots of ethyl ether. The extracts were pooled and the volume reduced to about 25 ml. The ether fraction was partitioned with 25 ml of 5% sodium bicarbonate. This process was repeated three more times, each time with 25 ml of

bicarbonate. Bicarbonate fraction was acidified to pH 3.0 with 6 N HCl and extracted three times with 50 ml of ethyl-ether. Ethyl ether was evaporated and the residue was dissolved in 2-3 ml of methanol and stored in a vial at 0°C in the refrigerator.

(b) Gibberellins

Two hundred fifty ml of saturated $NaHCO_3$ solution was mixed with 100 ml of culture filtrate in a separatory funnel. It was partitioned twice with 300 ml of ethyl acetate and the organic phase was discarded. The aquous layer was acidified to pH 2.5 using 5N HCl. Equal volume of ethyl acetate was added and shaken vigorously for 5 min in a separatory funnel. Extraction was done thrice with aquous layer using 300 ml of the solvent for each extraction, and all the ethyl acetate fractions were pooled and evaporated to dryness. The residue was dissolved in 1-2 ml of methanol.

(c) Cytokinins

Culture filtrate was acidified to pH 3.0 with 0.1N HCl, and extracted with diethyl ether thrice using a separatory funnel to remove the auxins. pH of the aqueous phase was then adjusted to 7.8 with 0.1N NH_4OH, and extracted thrice with equal volumes of n-butanol. The butanolic fractions were combined and evaporated. The residue was dissolved in 10 ml of 0.1N HCl and passed through a column of

Dowex-50 (H^+ Cycle). Cytokinins were eluted with 200 ml to dryness and the residue was dissolved in minimum quantity (1-2 ml) of distilled water. This extract was used for chromatography.

3) Chemical detection of growth hormones

(a) Auxins

Ascending paper chromatography was used to separate the indole compounds extracted from the fungal medium. 100 ul of the extract was placed on spots along a line 2.5 cm from the bottom of a 20 cm. square sheet of No.1 Whatman filter paper. The spots were dried with warm air using hair dryer. Spots of known indole compounds were placed on the chromatograms for reference. The edges of the filter paper were then stapled to make a cylinder, which was placed into the solvent, in a airtight-wide-mouth jar. The jar was then placed at room temperature in the dark for approximately six hours. The solvent used for separating the indole compounds was isopropyl alcohol, ammonia, water in the proportion 8:1:1. The Salkowski spray reagent as modified by Gordon and Weber (1951) contained 0.01 M $FeCl_3$ in 35% perchloric acid and diluted with an equal volume of absolute ethyl alcohol, was used to detect indole compounds on the chromatogram. The chromatogram was dried at room temperature and examined within 30 minutes of spraying.

(b) Gibberellins

Spots of the extract of mycorrhizal fungi on Whatman No.1 paper were developed ascendingly in the solvent such as isopropanol-ammonium hydroxide-water (10:1:1, v/v/v) and chromatogram was dried at room temperature. The chromatogram was then sprayed with either ethanol-conc. sulphuric acid (95:5, v/v) or Water-conc. H_2SO_4 (30:70, v/v), and dried at 120°C for 10 min in a hot air oven and then examined under UV light for fluorescent spots (Macmillan and Suter, 1963). Spots of known gibberellins were also run alongwith unknown samples to help identification.

(c) Cytokinins

Ascending paper chromatography was used to separate the cytokinin extract. 100 ul drops of each extract were run, using the freshly mixed solvent of isopropanol-ammonia-water (10:1:1, by volume). Cochromatography with standard of authentic substances was done for the identification of cytokinins. Chromatograms were dried and viewed under UV light and fluorescing spots were marked. Colour intensity, size of the spot and Rf values were the parameters used to express the results, qualitatively.

4) Quantitative measurement of growth hormones

(a) Auxins

One ml of the extract containing indole-compounds

was taken in a test tube and 2 ml of Salper reagent (1 ml of 0.5 M $FeCl_3$ was mixed in 50 ml of 35% (v/v) perchloric acid) was added dropwise but rapidly with continuous agitation. The sample was incubated in the dark for 60 minutes. The absorbance of the sample was measured in a spectrophotometer at 535 nm against a solvent-reagent blank, and the quantity of auxins in the extract was estimated from a standard curve drawn from known concentrations of IAA.

(b) Gibberellins

Two ml of the zinc acetate solution was added to 1 ml of the extract containing GA. After 2 min, 2 ml of potassium ferrocyanide solution (Holbrook et al., 1961) was mixed. The mixture was then centrifuged at low speed for 15 min. To 3 ml of the supernatant solution, 3 ml of 30 percent HCl was added in a test tube and the mixture was incubated at 20°C for 75 min. The absorbance of the sample and blank was measured at 254 nm in a spectrophotometer, and the amount of GA was calculated in the extract from a standard curve prepared with known GA_3 and the results were expressed as GA_3 equivalents.

(c) Cytokinins

To one ml of the extract, 2 ml of sulphuric acid was mixed, and the samples swere incubated at room temperature for 30 min. The absorbance of the mixture was taken in a

spectrophotometer at 254 nm against blank sample. The amount of cytokinins was quantified with known KI standard curve, and results were expressed as KI equivalents.

IV. Phosphorus uptake and phosphatase activity in orchids

(1) Raising of seedlings

Green pods of *Cymbidium elegans* and *C. giganteum*, were collected and surface sterilized by dipping them in absolute ethanol for a few seconds and followed by flamming (Linden, 1980). The seeds were then taken out in sterilized conditions and inoculated on the agar slopes of modified Knudson 'C' medium (Bose and Bhattacharjee, 1980), in 100 ml, 150 ml and 250 ml capacity conical flasks. Cultures were then placed at 25 ± 1°C, under 12 hr light and 12 hr dark conditions, in B.O.D. incubators. After 3 months of growth, germinated protocorms were again subcultured on freshly prepared nutrient medium, upto 9 months.

(2) Preparation of mycorrhizal inoculum

The orchid mycorrhizal fungus, i.e., RH 46, was cultured on Pfeffer mineral liquid medium (Hadley and Ong, 1978), in conical flasks and incubated at 25 ± 1°C, in the dark for 10-12 days. Fungus grown in liquid medium was filtered through Whatman filter paper No. 1. The mycelium of the fungus was homogenized. 20 ml of homogenate was

inoculated per pot to the roots of seedlings to produce mycorrhizal plants.

(3) Preparation of potting medium and treatments

The air dried cowdung, garden soil, and charcoal powder (1:1:0.4) were mixed to form basal substrate-medium for growing seedlings, in glass house conditions. Phosphorus as KH_2PO_4 was applied at five levels, viz., 1.68 mg, 3.36 mg, 33.6 mg, 67.2 mg and 0.00 mg in the 150 gm soil mixtured thoroughly and filled in separate earthen community pots (10 cm diameter), having a hole at the bottom. 1-2 cm thick layer of sterilized stones was added, in all the pots before filling the mixture, the substrate filled pots were sterilized twice in autoclave for 30 min. at 15 p.s.i. 30 replicates were made for each treatment using each species.

(4) Transplantation of seedlings to earthen pots

Two to three leaf stage (9 months old) seedlings of each species were carefully removed from the flasks. The roots of the seedlings were gently washed in running tap water. Six-washed seedlings were then transplanted to each community pot. Earthen pots were kept at 16.5°C - 26°C range of temperature in the glass house. Seedlings were watered daily (50 ml/pot) with tap water. The mycorrhizal and non-mycorrhizal seedlings grown at different levels of phosphorus in the soil

mixture, were harvested at monthly intervals, and were used to study phosphorus uptake and phosphatase activity.

(5) Determination of total phosphorus

Phosphorus was determined using Vanado-molybdic Yellow colour method (Jackson, 1967). At monthly intervals, seedlings were harvested and washed carefully in running tap water, and the fresh and dry weight was calculated. Oven-dried ground plant samples were taken in 250 ml conical flasks and 10 ml of triacid mixture was added. The digestion was done using hot plate at 200 - 300°C for 1 - 2 hours, and the residue was diluted with little water. The residue was then filtered through ordinary filter paper, in 50 ml capacity volumetric flasks, and then volume was made to 50 ml with distilled water. To 40 ml of digested sample, 10 ml of ammonium vanado molybdate solution was mixed and the total volume was made to 50 ml. The mixture was kept for 30 min at room temperature to develop maximum colour and the absorbance was measured at 490 nm using spectrophotometer.

(6) Quantitative analysis of phosphatases

The soluble root enzymes were extracted by macerating the seedlings (2:1, w/v) in a mortar at 5°C using 0.1M borate buffer (pH 8.8) + 0.1% glutathione. The macerate was then centrifuged at 20,000 x g for 20 min and the acid and

alkaline phosphatase activity in the supernatant was determined quantitatively measuring the amount of p-nitrophenol (PNP) released by the enzyme from p-nitrophenyl phosphate (Gianinazzi et al., 1976).

RESULTS.

I. Effect of Temperature, pH and Light on Asymbiotic Seed Germination and subsequent Seedling Growth of Orchids

1) Effect of temperature

Effect of temperature on seed germination and seedling development of orchids in asymbiotic conditions varied (Figs. 3 and 4, Table 1, Plate 5). Maximum germination of seeds in case of *Cymbidium elegans* was observed at 20°C whereas in *C. giganteum* and *Thunia alba* maximum germination was recorded at 25°C (Fig. 3). Higher temperatures were inhibitory. Minimum seed germination was noticed at 35°C temperature in all the species (Fig. 3). Growth of seedlings in *C. elegans* was highest at 20°C, whereas other temperature treatments resulted in poor growth. The highest growth index of seedling development in *C. elegans* was recorded at 25°C temperature while the lowest was at 35°C (Fig. 4). Similarly, growth index was higher at 25°C in *C. giganteum* and *T. alba* (Fig. 4). Seedlings/plantlets were formed at all the temperatures but a considerable reduction in area of seedlings/plantlets was obtained at 35°C (Table 1). Maximum number and area of 150-day-old seedlings/plantlets occurred at 20°C in *C. elegans* followed by *C. giganteum* at 25°C and at 30°C in case of *T. alba* (Table 1). Leaf formation and its development also varied at different temperatures. Leaf primordia/leaves were developed in

Fig. 3 : Seed germination (%) of *Cymbidium elegans*, *C. giganteum* and *Thunia alba* at different temperatures.

Fig. 3

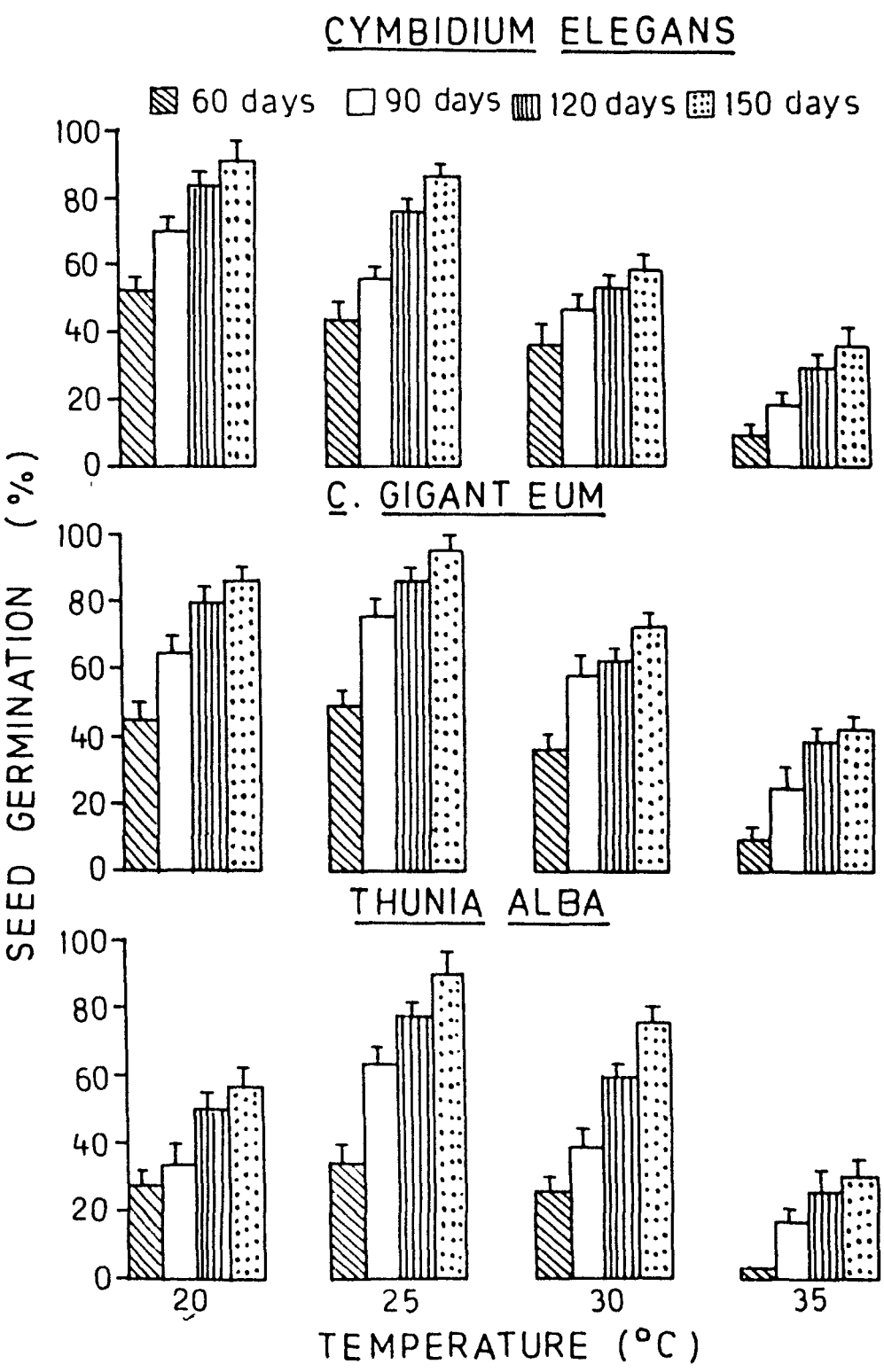

CYMBIDIUM ELEGANS
60 days
90 days
120 days
150 days
SEED GERMINATION (%)
100
80
60
40
20
0
C. GIGANT EUM
THUNIA ALBA
20
25
30
35
TEMPERATURE (°C)

Fig. 4 : Seedling growth index of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, at different temperatures.

Fig.4

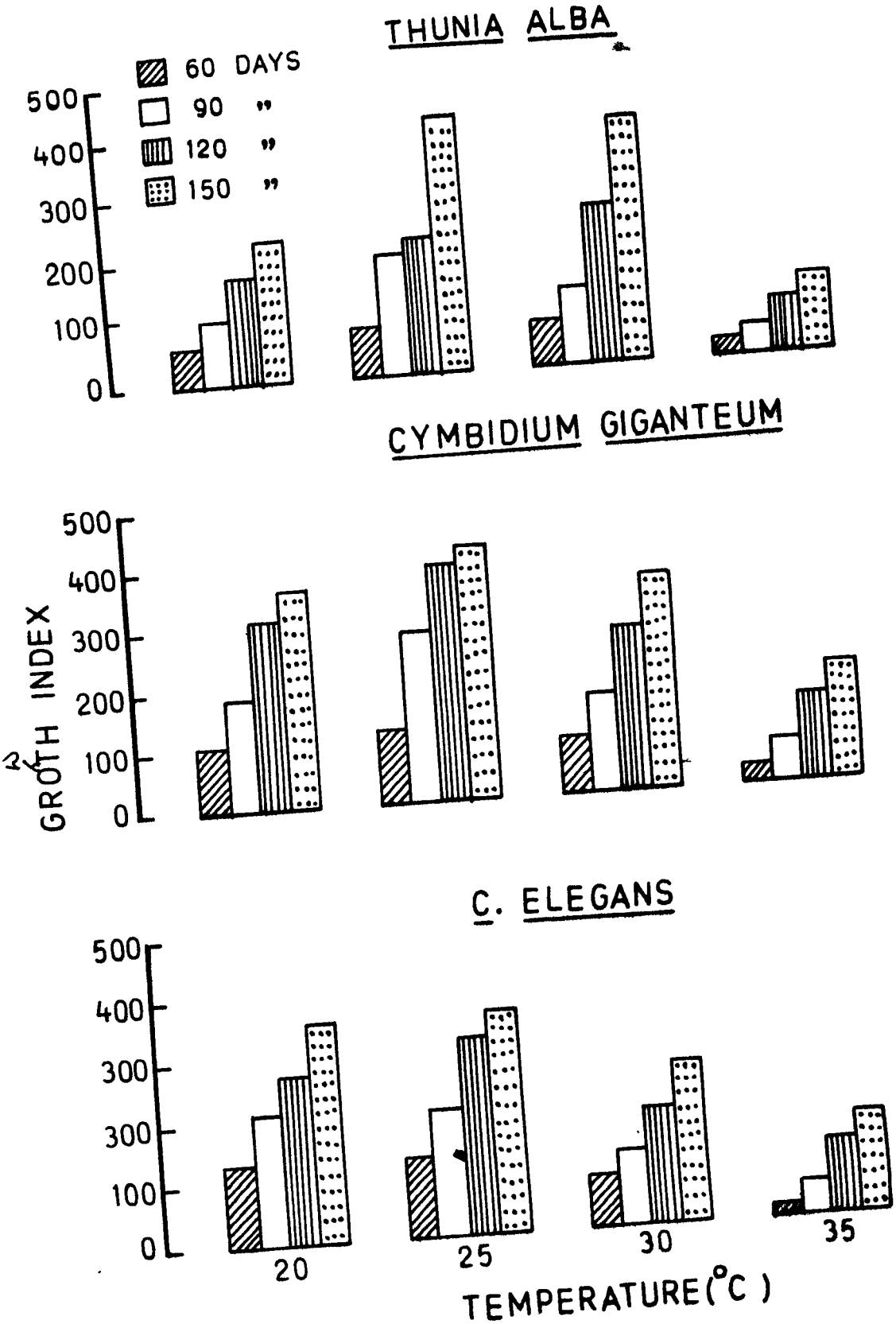

THUNIA ALBA
60 DAYS
90 "
120 "
150 "
500
400
300
200
100
0
CYMBIDIUM GIGANTEUM
GROWTH INDEX
C. ELEGANS
20
25
30
35
TEMPERATURE(°C)

Table 1 : Effect of temperature on the growth of seedlings/ plantlets of orchids in asymbiotic conditions

Orchid species	Culture period (days)	Average area of seedlings/plantlets (mm^2)			
		20°C	25°C	30°C	35°C
C. elegans	60	0.36	0.25	0.04	0.01
	90	1.08	0.64	0.22	0.15
	120	2.69	3.08	1.35	0.39
	150	5.87	3.82	2.66	1.72
C. giganteum	60	0.29	1.27	0.25	0.17
	90	2.20	7.09	0.92	0.22
	120	5.50	17.13	4.86	1.75
	150	15.96	25.20	15.24	10.15
T. alba	60	0.06	0.19	0.10	-
	90	1.27	1.00	1.40	1.06
	120	1.85	14.46	14.60	1.27
	150	13.93	15.21	16.17	4.22

Table 2 : Effect of temperature on the production of leaf primordia/leaves of orchids in asymbiotic conditions

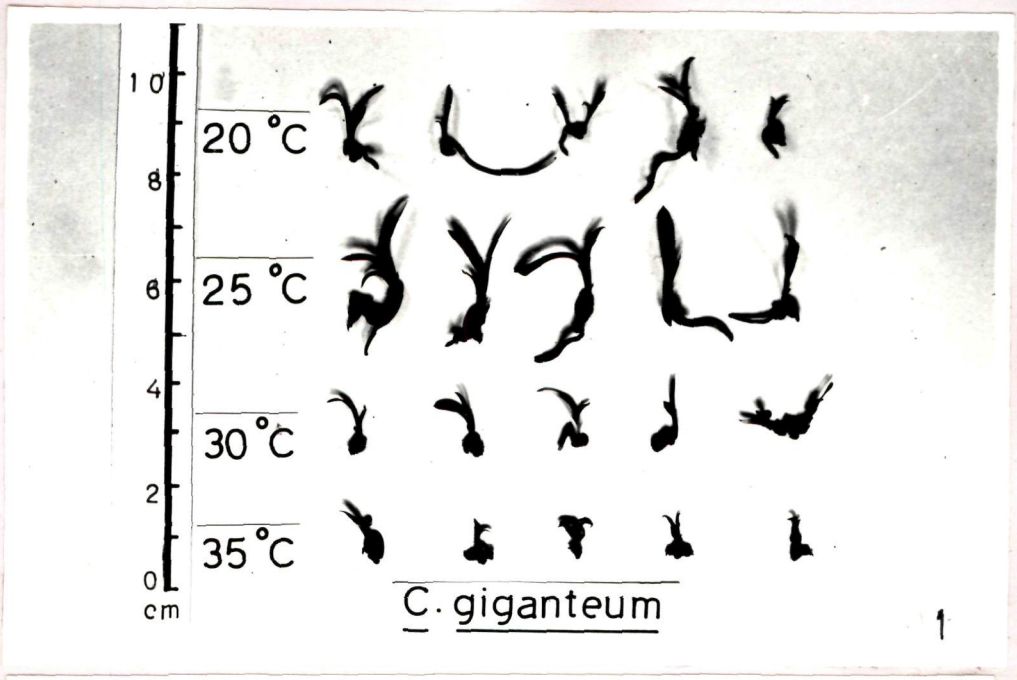
Orchid species	Culture period (days)	Average No. and area of leaf primordia/leaves (mm^2)							
		20°C		25°C		30°C		35°C	
		No.	area	No.	area	No.	area	No.	area
C. elegans	60	1.14	0.06	0.94	0.03	-	-	-	-
	90	1.42	0.19	1.28	0.14	0.78	0.02	0.63	0.01
	120	1.91	0.64	1.96	0.91	1.50	0.22	0.92	0.02
	150	2.37	1.82	2.15	1.07	1.83	0.77	1.37	0.42
C. giganteum	60	-	-	0.95	0.11	-	-	-	-
	90	1.35	0.13	1.95	1.12	0.76	0.05	-	-
	120	1.74	0.84	2.52	2.25	1.97	0.86	1.01	0.08
	150	2.15	1.69	2.75	2.95	2.42	1.80	1.52	0.69
T. alba	60	-	-	0.86	0.02	-	-	-	-
	90	0.97	0.04	1.46	0.34	1.54	0.08	-	-
	120	1.35	0.29	2.14	1.84	2.49	2.32	0.83	0.01
	150	1.82	1.50	2.29	2.17	2.83	3.03	1.37	0.60

Table 3 : Effect of temperature on the production of rhizoids/roots of orchids in asymbiotic conditions

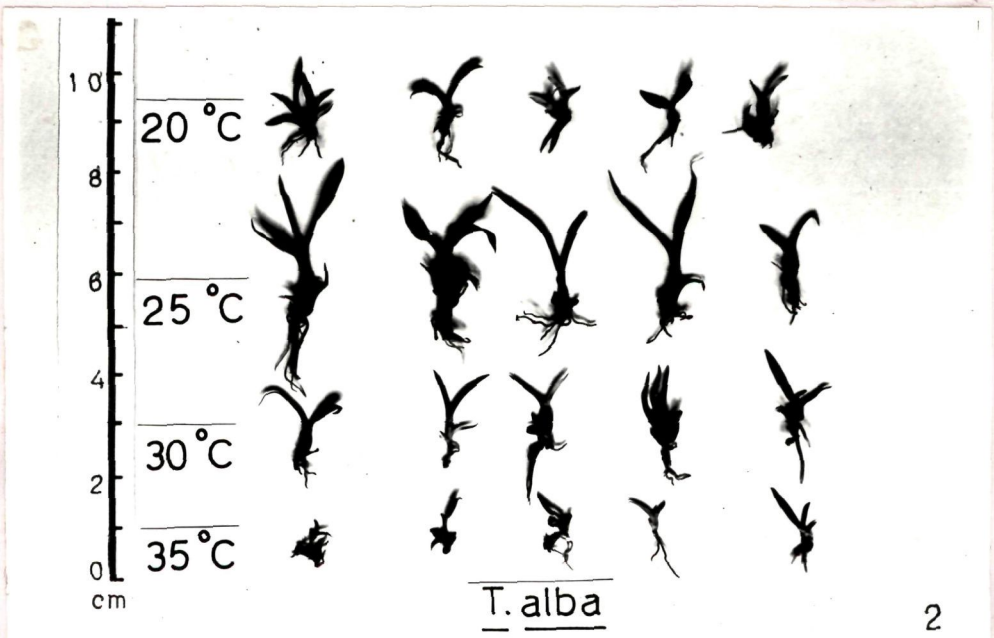
Orchid species	Culture period (days)	Average number and area of rhizoids/roots (mm^2)							
		20°C		25°C		30°C		35°C	
		No.	area	No.	area	No.	area	No.	area
C. elegans	60	6.88	0.003	5.45	0.001	6.28	0.0003	-	-
	90	14.37	0.007	8.96	0.004	8.44	0.001	8.59	0.0007
	120	17.32	0.104	11.65	0.22	10.10	0.02	9.05	0.004
	150	19.69	0.41	13.07	0.30	12.74	0.16	10.00	0.13
C. giganteum	60	5.62	0.0009	5.80	0.002	4.55	0.0005	2.87	0.0003
	90	9.01	0.004	10.13	0.22	8.15	0.002	5.76	0.0005
	120	9.05	0.12	10.39	1.16	10.42	0.13	6.68	0.004
	150	9.82	0.51	12.06	1.38	11.31	0.55	8.30	0.42
T. alba	60	0.28	0.0009	0.80	0.001	0.32	0.001	-	-
	90	1.05	0.001	1.41	0.06	0.85	0.003	0.66	0.0008
	120	1.55	0.04	2.10	0.29	1.43	0.13	1.07	0.005
	150	1.98	0.23	2.81	0.37	1.89	0.36	1.85	0.17

Plate 5 : Seedling growth of C. giganteum (Fig. 1), T. alba (Fig. 2) and C. elegans (Fig. 3), at different temperatures.

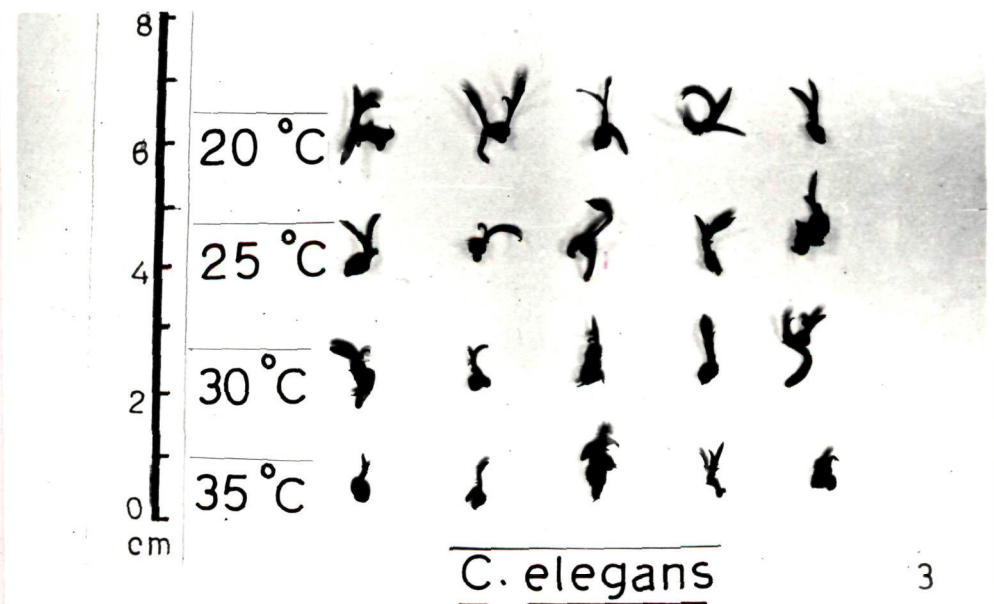
PLATE 5



C. giganteum 1



T. alba 2



C. elegans 3

60-day-old seedlings at 20 and 25°C temperatures in C. elegans. However, in C. giganteum and T. alba leaf primordia/leaves were produced at 25°C only after 60 days (Table 2). Minimum number and area of leaf primordia/leaves was observed at 35°C in all the species (Table 2).

The largest number and area of rhizoids/roots were recorded at 20°C in C. elegans followed by C. giganteum and T. alba at 25°C (Table 3). The seedling growth was inhibited at 35°C (Tables 1-3 and Plate 5). Seedlings/plantlets showed poor growth and turned brown at 35°C after 150 days (Plate 5).

Statistically significant variations were obtained between different temperatures and the ages of culture in relation to the seed germination and seedling growth in all the species at 1% level.

2) Effect of pH of the medium

The results showed that the germination of seeds and their subsequent growth varied at different pH levels (Figs. 5 and 6, Tables 4-6 and Plate 6). The highest germination was found at pH 4.0, 5.0 and 6.0 in C. elegans, C. giganteum and T. alba, respectively. Whereas, lowest germination occurred at pH 10.0 in all the species (Fig. 5). Significant variations were observed at 1% level in seed germination between different pH values.

Fig. 5 : Seed germination (%) of *Cymbidium elegans*, *C. giganteum* and *Thunia alba* at different pH levels of the medium.

Fig.5

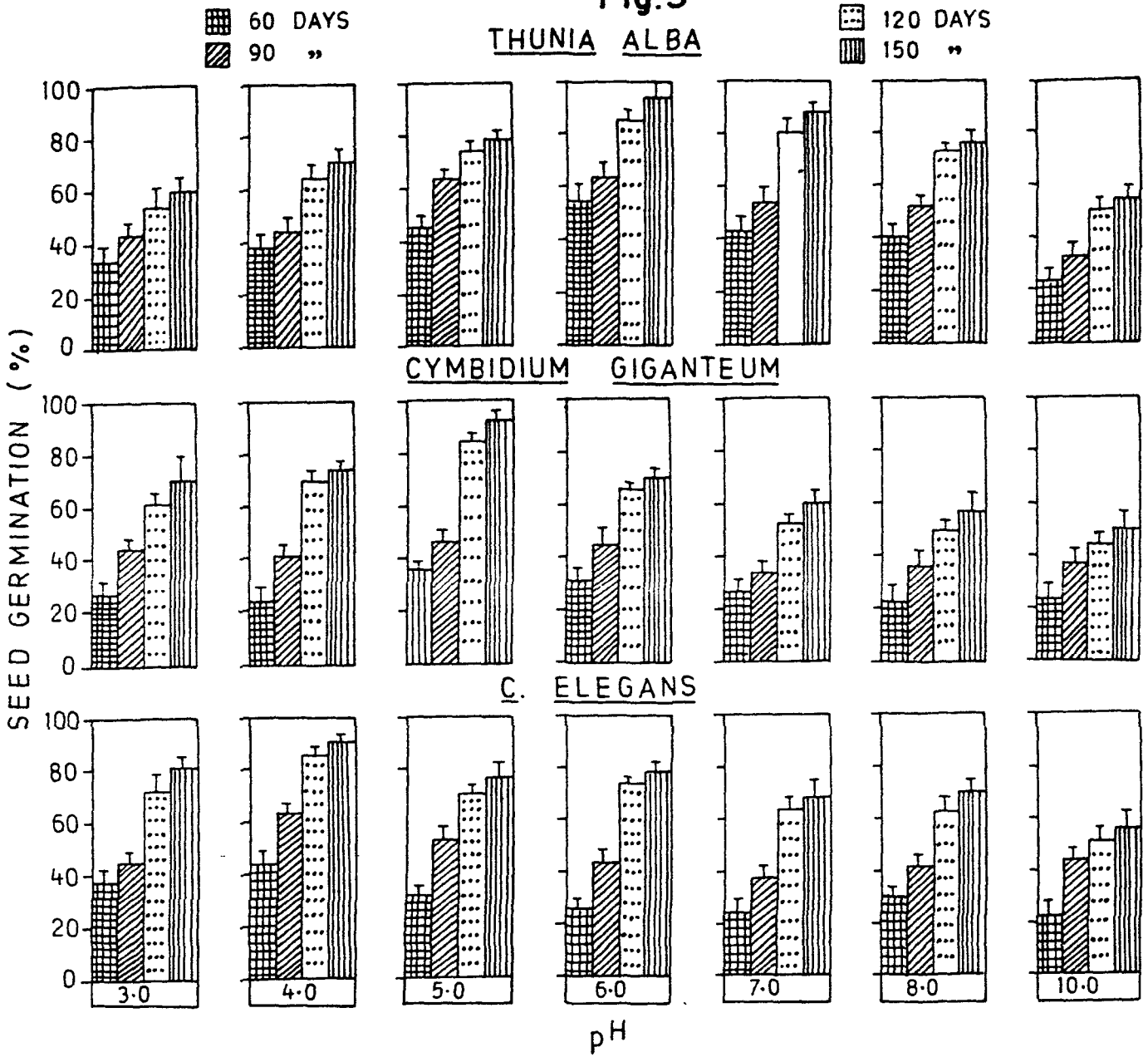

60 DAYS
90 "
120 DAYS
150 "
THUNIA ALBA
CYMBIDIUM GIGANTEUM
C. ELEGANS
SEED GERMINATION (%)
100
80
60
40
20
0
3·0
4·0
5·0
6·0
7·0
8·0
10·0
pH

Fig. 6: Seedling growth index of *Cymbidium elegans*, *C. giganteum* and *Thunia alba* at different pH levels of the medium.

Fig 6

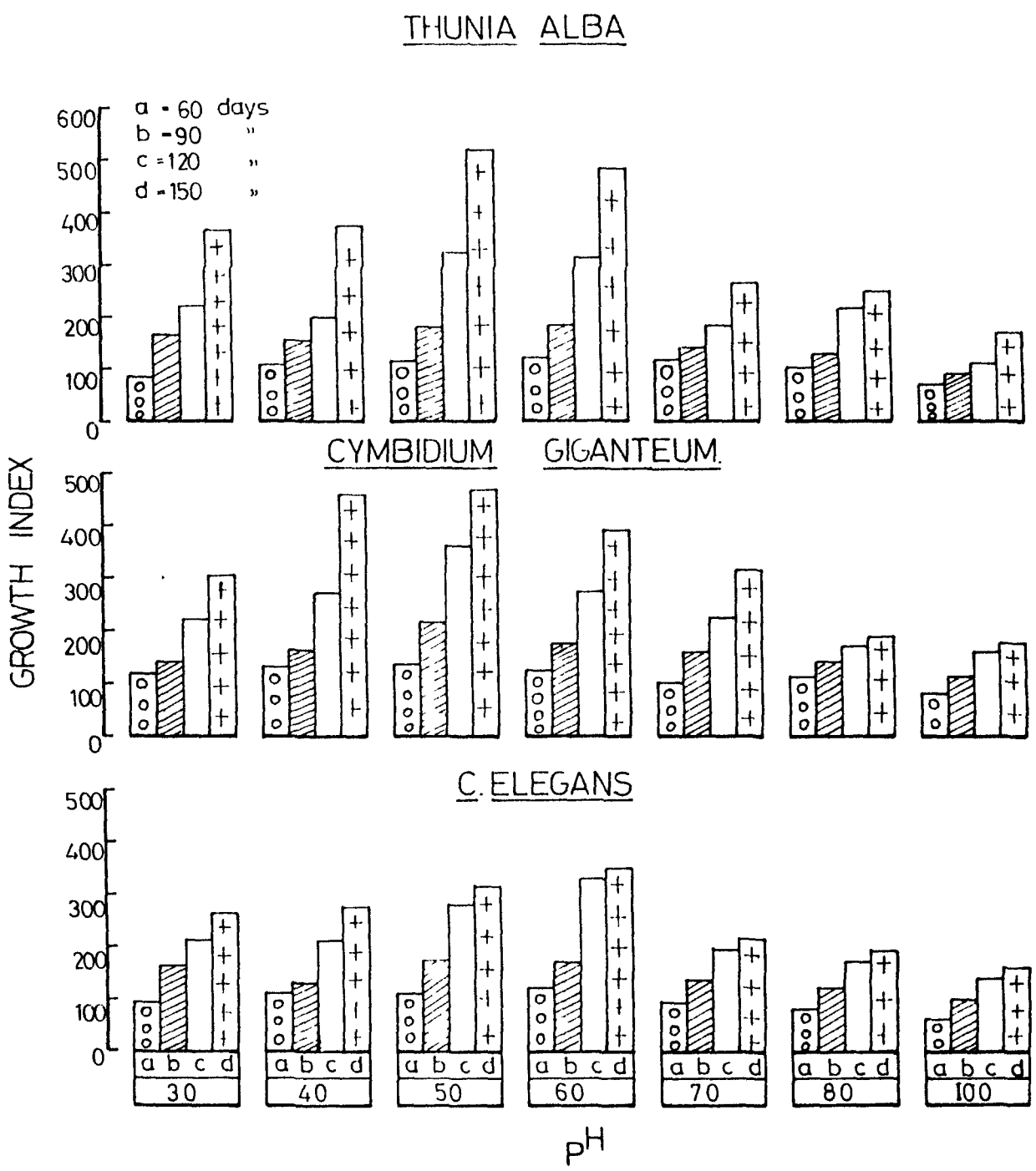

THUNIA ALBA
a - 60 days
b - 90 "
c - 120 "
d - 150 "
600
500
400
300
200
100
0
CYMBIDIUM GIGANTEUM.
GROWTH INDEX
500
400
300
200
100
0
C. ELEGANS
500
400
300
200
100
0
a b c d
30
40
50
60
70
80
100
pH

Table 4 : Effect of pH of the medium on the growth of seedlings/plantlets of orchids in asymbiotic conditions

Orchid species	Culture period (days)	Average area of seedlings/plants (mm^2)						
		3.0	4.0	5.0	6.0	7.0	8.0	10.0
C. elegans	60	0.04	0.05	0.28	0.23	0.04	0.02	0.01
	90	0.48	0.56	1.02	1.97	0.38	0.39	0.11
	120	1.50	1.51	5.29	4.40	1.12	1.08	0.70
	150	3.07	3.78	6.75	5.69	2.65	2.35	1.66
C. giganteum	60	0.24	0.91	1.24	1.04	0.79	0.17	0.09
	90	2.09	2.33	6.67	2.60	2.12	0.74	0.45
	120	14.44	15.82	19.97	15.15	13.04	4.53	3.69
	150	16.36	17.82	23.55	17.16	14.31	14.31	13.58
T. alba	60	0.05	0.13	0.23	0.17	0.05	0.02	0.03
	90	0.23	0.25	1.26	0.32	0.24	0.17	0.07
	120	3.86	4.46	6.05	4.56	3.48	2.41	1.39
	150	14.22	15.04	16.34	14.79	13.92	10.29	10.79

Table 5 : Effect of pH of the medium on the production of leaf primordia/leaves of orchids in asymbiotic conditions

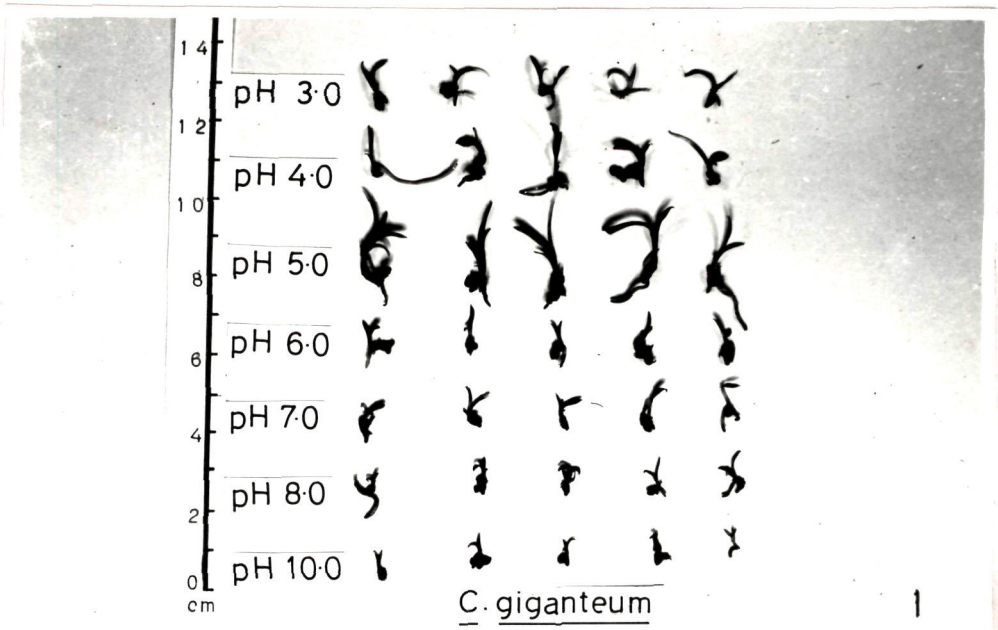
Orchid species	Culture period (days)	Average number and area of leaf primordia/leaves (mm^2)													
		3.0		4.0		5.0		6.0		7.0		8.0		10.0	
		No.	area	No.	area	No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	-	-	-	-	1.12	0.03	1.05	0.02	-	-	-	-	-	-
	90	1.13	0.09	1.42	0.18	1.31	0.46	1.28	0.20	0.94	0.08	0.83	0.06	0.45	0.01
	120	1.74	0.23	1.92	0.64	1.85	0.90	1.68	0.75	1.17	0.19	1.08	0.15	0.87	0.12
	150	2.07	0.71	2.42	1.81	1.95	1.03	1.80	0.80	1.43	0.69	1.28	0.50	1.08	0.32
C. giganteum	60	-	-	0.85	0.06	0.97	0.11	0.63	0.09	0.51	0.07	-	-	-	-
	90	1.15	0.13	1.37	0.14	1.81	1.07	1.09	0.20	0.92	0.16	0.56	0.07	0.44	0.04
	120	1.98	1.41	2.15	1.49	2.44	2.31	1.69	1.59	1.57	1.31	1.10	0.73	0.94	0.38
	150	2.11	1.54	2.26	1.74	2.66	3.08	1.88	1.92	1.69	1.55	1.67	1.26	1.23	0.86
T. alba	60	-	-	0.65	0.01	0.79	0.01	1.12	0.03	-	-	-	-	-	-
	90	0.72	0.03	1.02	0.05	1.47	0.35	1.57	0.09	1.34	0.01	1.07	0.01	0.82	0.002
	120	1.46	0.77	1.72	1.24	2.02	1.74	2.47	2.33	2.00	0.56	1.55	0.39	1.19	0.06
	150	1.58	0.91	1.80	1.33	2.15	1.96	2.65	3.16	2.08	0.67	1.66	0.41	1.41	0.23

Table 6 : Effect of pH of the medium on the production of rhizoids/roots of orchids in asymbiotic conditions

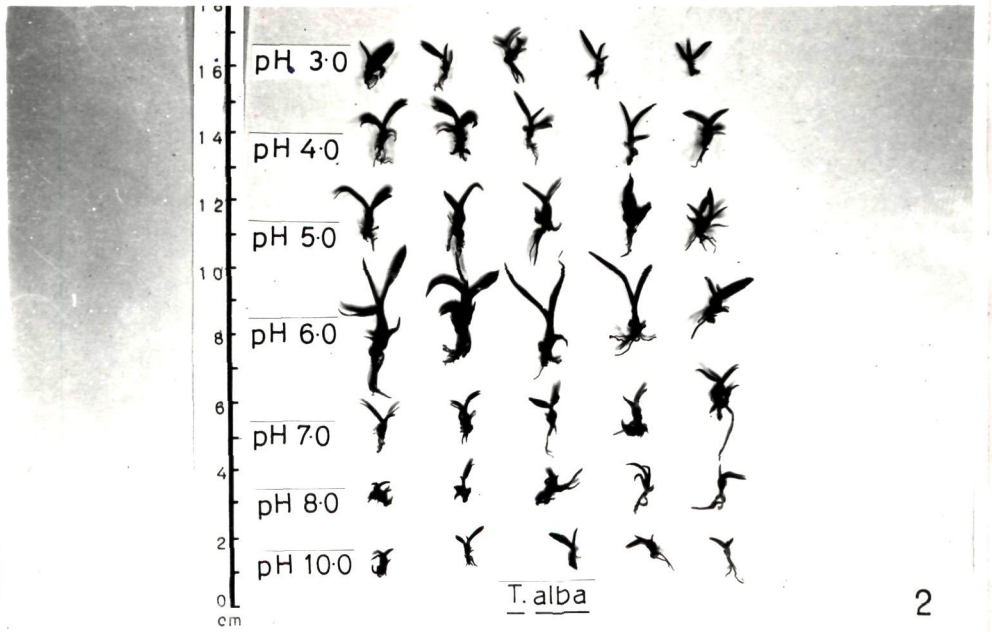
Orchid species	Culture period (days)	Average number and area of rhizoids/roots (mm^2)													
		3.0		4.0		5.0		6.0		7.0		8.0		10.0	
		No.	area	No.	area	No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	2.86	0.0008	3.95	0.001	4.15	0.002	3.40	0.001	3.25	0.001	2.75	0.0007	1.88	0.0004
	90	8.99	0.004	10.11	0.006	8.48	0.004	5.70	0.02	5.84	0.002	4.72	0.002	3.80	0.001
	120	11.00	0.05	12.74	0.06	11.27	0.22	8.06	0.03	8.86	0.02	6.88	0.01	5.66	0.02
	150	11.73	0.24	14.20	0.33	13.14	0.26	11.78	0.20	9.98	0.13	7.92	0.10	7.46	0.09
C. giganteum	60	4.27	0.0006	8.72	0.001	7.12	0.002	7.08	0.001	8.61	0.001	3.15	0.001	2.26	0.001
	90	11.26	0.004	9.25	0.004	11.43	0.20	11.17	0.004	9.51	0.003	8.36	0.003	6.18	0.002
	120	13.16	0.31	10.89	0.48	13.95	1.07	11.19	0.93	9.83	0.54	9.20	0.13	7.73	0.11
	150	14.19	0.04	12.78	0.68	15.28	1.71	13.14	1.17	12.09	1.14	10.50	0.56	8.26	0.25
P. alba	60	0.31	0.0006	0.79	0.001	0.88	0.001	1.02	0.002	0.37	0.0007	0.31	0.0005	0.23	0.0002
	90	1.27	0.06	1.36	0.003	1.62	0.12	1.45	0.007	1.10	0.003	0.86	0.003	0.53	0.001
	120	1.88	0.40	2.15	0.37	2.50	0.57	2.60	0.71	1.37	0.40	1.50	0.23	0.99	0.03
	150	2.08	0.52	2.43	0.66	2.78	0.85	3.18	1.38	1.95	0.51	1.64	0.31	1.27	0.11

Plate 6 : Seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), at different pH levels of the medium.

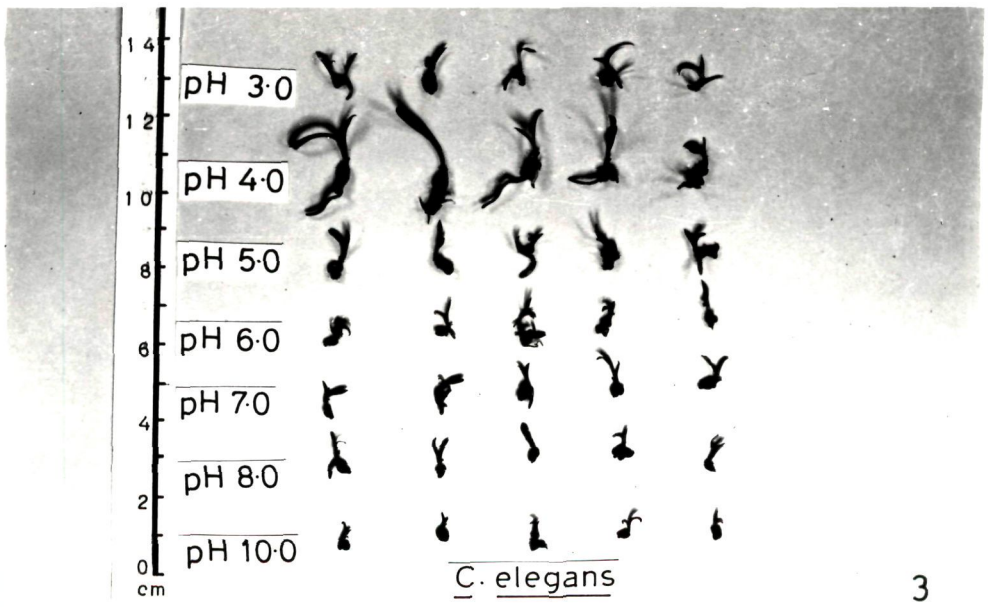
PLATE 6



C. giganteum 1



T. alba 2



C. elegans 3

Seedling growth was significantly higher between the pH range of 5.0 - 6.0 (Fig. 6, Table 4). Maximum growth index of seedling development was noticed at pH 6.0 in C. elegans whereas, C. giganteum and T. alba exhibited the highest growth index at pH 5.0 (Fig. 6). Statistically significant variations were obtained at 5% level between various pH and at 1% level between ages of cultures in relation to the growth index in all the species.

The highest area of seedlings/plantlets was noticed at pH 5.0 and lowest at pH 10.0 in all the cases (Table 4). Analysis of variance showed significant variations at 1% level between different culture periods in relation to the area of seedlings/plantlets but the average area of seedlings/plantlets was statistically insignificant at different pH values.

Development of leaf primordia/leaves also varied at different pH levels. The maximum average number and area of leaf primordia/leaves were observed at pH 4.0, 5.0 and 6.0 in C. elegans, C. giganteum and T. alba, respectively. However, leaf development was highly inhibited at pH 10.0 (Table 5). The development of rhizoids/roots was comparatively better between pH range of 4.0 - 6.0 in all the species. The largest number and area of rhizoids/roots were recorded at pH 5.0 in C. giganteum followed by C. elegans at pH 4.0 and T. alba at pH 6.0 (Table 6). The overall growth of seedlings, roots and leaves was suppressed at pH 8.0 and 10.0 in all the species

(Plate 6).

3) Effect of light

a) Effect of light intensity

The seed germination and subsequent seedling growth of all the orchid species was greatly influenced by different light intensities (Plate 7, Figs. 7 and 8). The maximum seed germination was observed in dark in case of C. elegans and T. alba. On the other hand, C. giganteum showed higher percentage of seed germination at 1500 lux light. However, the lowest seed germination occurred at 5000 lux light (Fig. 7). The seed germination showed significant variation at 1% level between different light intensities in all the species. The growth index was maximum at 1500 lux in C. elegans and at 3000 lux in C. giganteum and T. alba while the lowest growth index occurred under dark conditions in all the cases (Fig. 8).

Significant variations were observed between different light intensities and culture periods at 1% level in relation to the growth index in all the orchid species. Similarly, the maximum area of seedlings/plantlets was also achieved at 1500 lux in C. elegans and at 3000 lux in C. giganteum and T. alba whereas, the minimum area of seedlings/plantlets was recorded in the dark conditions (Table 7).

Fig. 7 : Seed germination (%) of *Cymbidium elegans* *C. giganteum* and *Thunia alba* at different light intensities.

Fig. 7

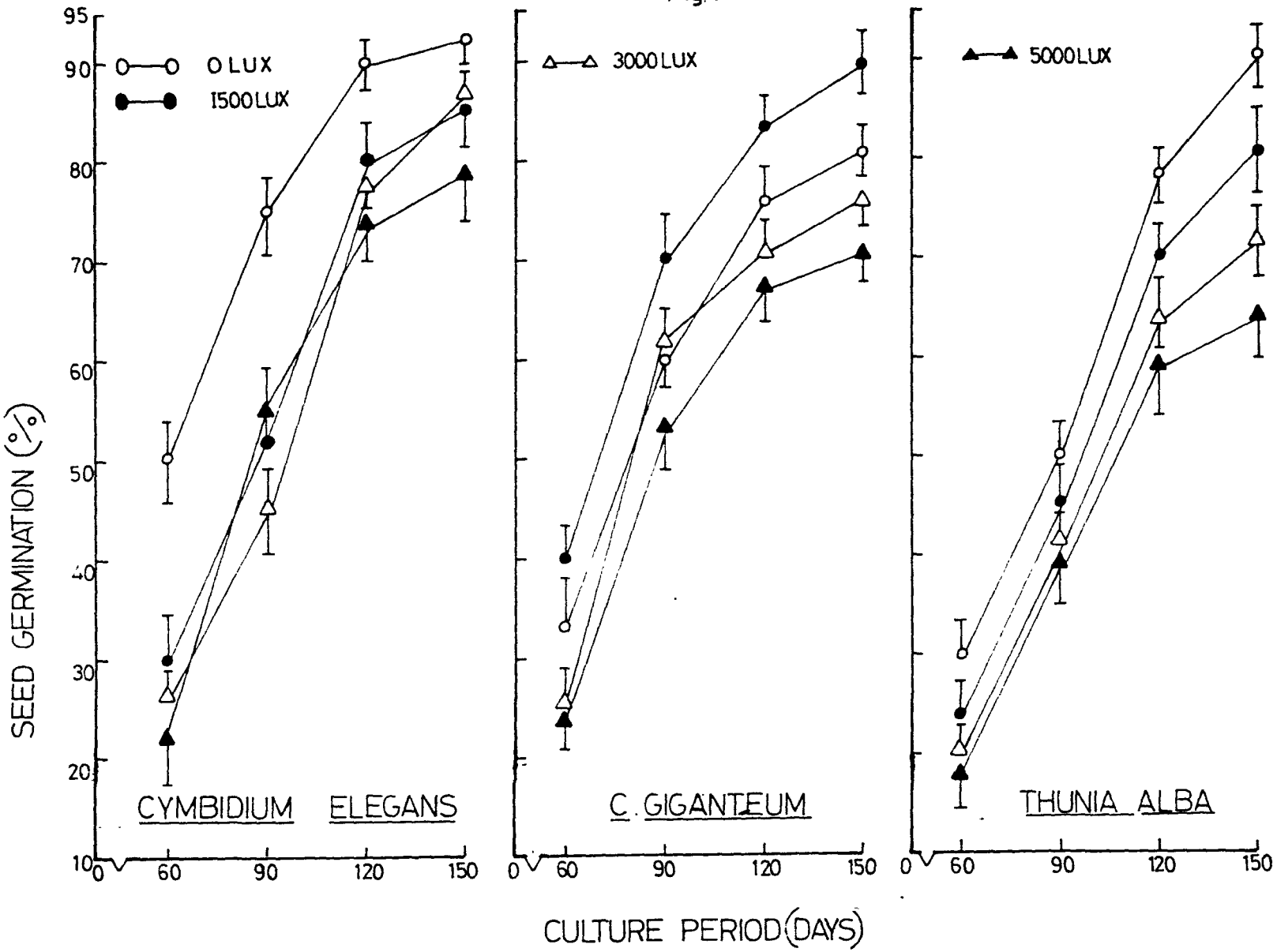

SEED GERMINATION (%)
95
90
80
70
60
50
40
30
20
10
0 LUX
1500 LUX
3000 LUX
5000 LUX
CYMBIDIUM ELEGANS
C. GIGANTEUM
THUNIA ALBA
0
60
90
120
150
CULTURE PERIOD (DAYS)

Fig. 8 : Seedling growth index of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, at different light intensities.

Fig: 8

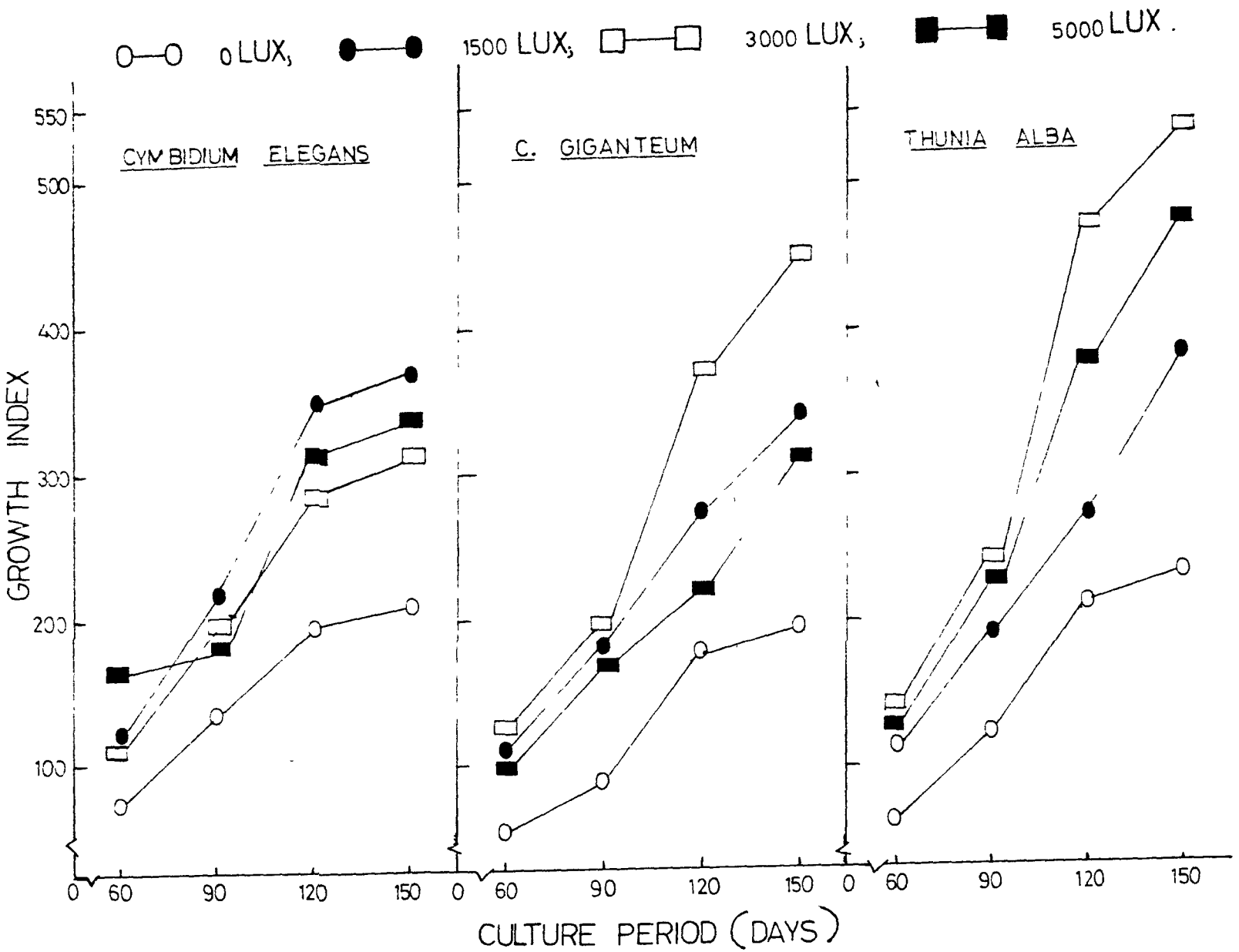

0 LUX,
1500 LUX,
3000 LUX,
5000 LUX.
CYMBIDIUM ELEGANS
C. GIGANTEUM
THUNIA ALBA
GROWTH INDEX
550
500
400
300
200
100
0
60
90
120
150
CULTURE PERIOD (DAYS)

Table 7 : Effect of light intensities on the growth of seedlings/ plantlets of orchids in asymbiotic conditions

Orchid species	Culture Period (days)	Average area of seedlings/plantlets (mm^2)			
		0 lux	1500 lux	3000 lux	5000 lux
C. elegans	60	0.03	0.26	0.16	0.07
	90	0.12	1.01	0.63	0.18
	120	0.41	5.32	3.99	1.66
	150	2.52	7.24	5.08	4.13
C. giganteum	60	0.11	0.22	1.04	0.66
	90	0.54	2.12	6.51	1.94
	120	3.63	14.48	19.82	11.61
	150	7.87	17.07	23.18	14.05
T. alba	60	0.02	0.04	0.18	0.11
	90	0.06	0.20	1.09	0.23
	120	1.37	3.52	5.67	3.84
	150	4.33	14.26	17.21	15.26

Table 8 : Effect of light intensities on the production of leaf primordia/leaves of orchids in asymbiotic conditions

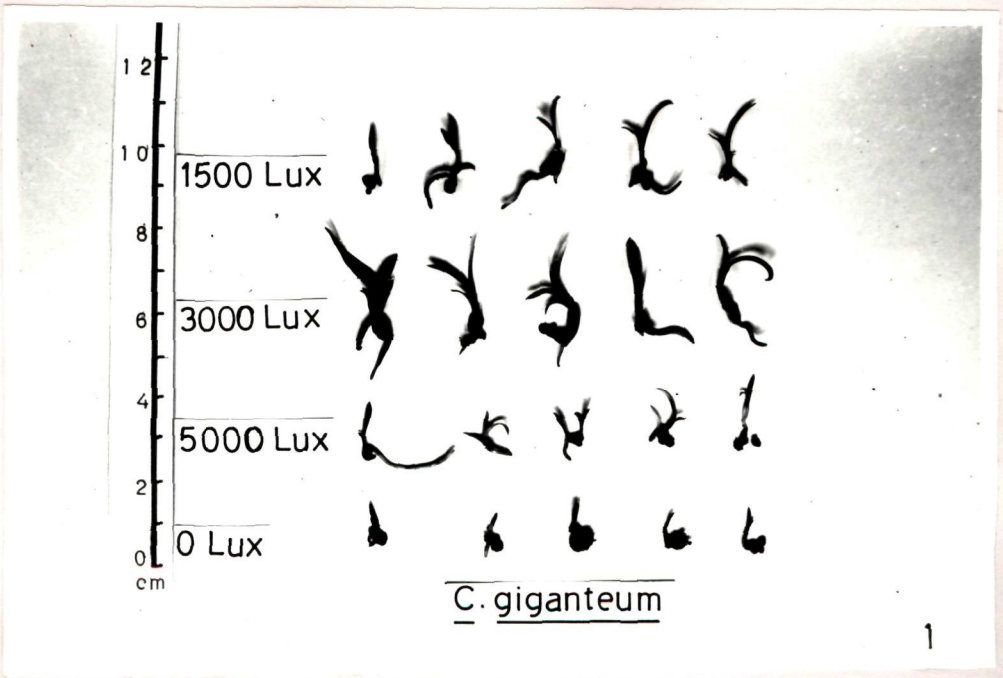
Orchid species	Culture period (days)	Average number and area of leaf primordia/leaves (mm^2)							
		0 lux		1500 lux		3000 lux		5000 lux	
		No.	area	No.	area	No.	area	No.	area
C. elegans	60	-	-	1.14	0.06	1.06	0.03	-	-
	90	0.76	0.01	1.58	0.17	1.29	0.09	1.12	0.02
	120	1.04	0.04	2.26	1.71	1.85	0.98	1.35	0.18
	150	1.31	0.22	2.37	2.06	1.97	1.08	1.63	0.66
C. giganteum	60	-	-	-	-	0.89	0.10	0.92	0.07
	90	0.58	0.05	0.95	0.13	1.65	1.00	1.28	0.14
	120	1.17	0.38	1.61	1.38	2.42	2.28	1.75	1.10
	150	1.37	0.76	1.71	1.56	2.70	2.75	1.82	1.86
T. alba	60	-	-	-	-	1.07	0.04	0.87	0.02
	90	0.57	0.005	0.96	0.04	1.83	0.62	1.40	0.06
	120	1.13	0.08	1.67	0.94	2.52	2.85	1.96	1.45
	150	1.39	0.36	1.78	1.09	2.78	3.28	2.13	1.64

Table 9 : Effect of light intensities on the production of rhizoids/roots of orchids in asymbiotic conditions

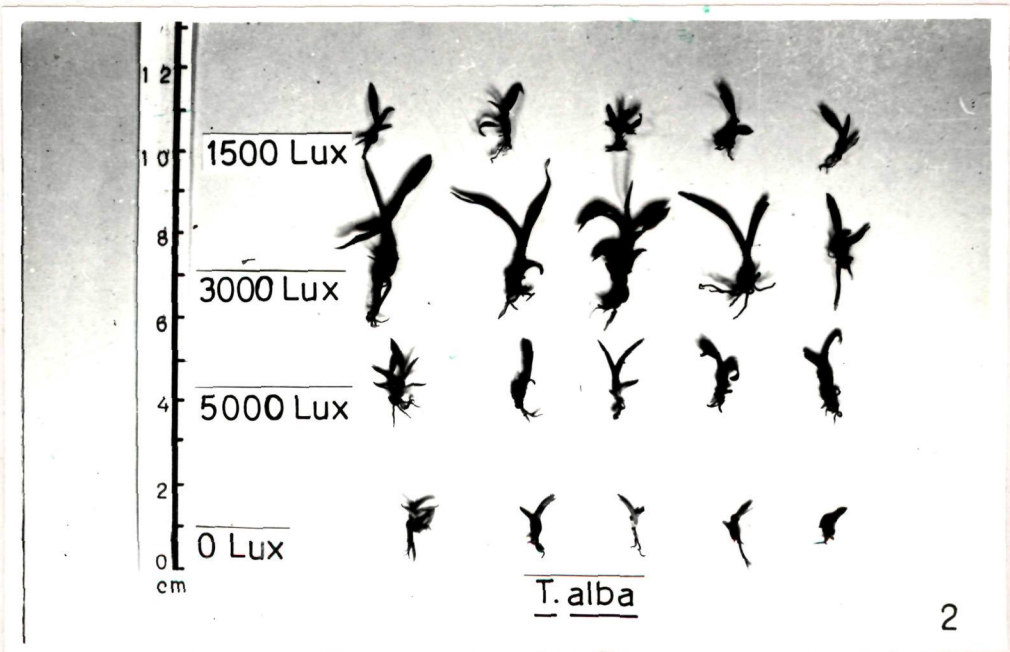
Orchid species	Culture period (days)	Average number and area of rhizoids/roots (cm^2)							
		0 lux		1500 lux		3000 lux		5000 lux	
		No.	area	No.	area	No.	area	No.	area
C. elegans	60	4.45	0.002	4.88	0.002	5.51	0.002	3.65	0.001
	90	5.37	0.003	10.91	0.005	6.82	0.004	7.49	0.082
	120	8.92	0.004	12.32	0.28	7.58	0.26	8.03	0.025
	150	10.02	0.11	13.14	0.34	10.22	0.27	8.16	0.17
C. giganteum	60	4.18	0.002	4.13	0.001	9.01	0.001	3.65	0.001
	90	7.67	0.003	12.12	0.004	9.86	0.16	7.67	0.003
	120	11.15	0.04	13.91	0.70	10.53	0.75	8.11	0.40
	150	12.06	0.16	15.39	1.16	11.95	0.86	10.02	0.46
T. alba	60	0.71	0.001	0.51	0.0009	0.95	0.001	0.75	0.001
	90	1.37	0.003	1.27	0.003	1.69	0.18	1.42	0.002
	120	1.65	0.03	2.06	0.33	2.55	0.72	2.03	0.18
	150	1.78	0.17	2.33	0.47	2.82	0.83	2.15	0.46

Plate 7 : Seedling growth of C. giganteum (Fig. 1), T. alba (Fig. 2) and C. elegans (Fig. 3), at different light intensities.

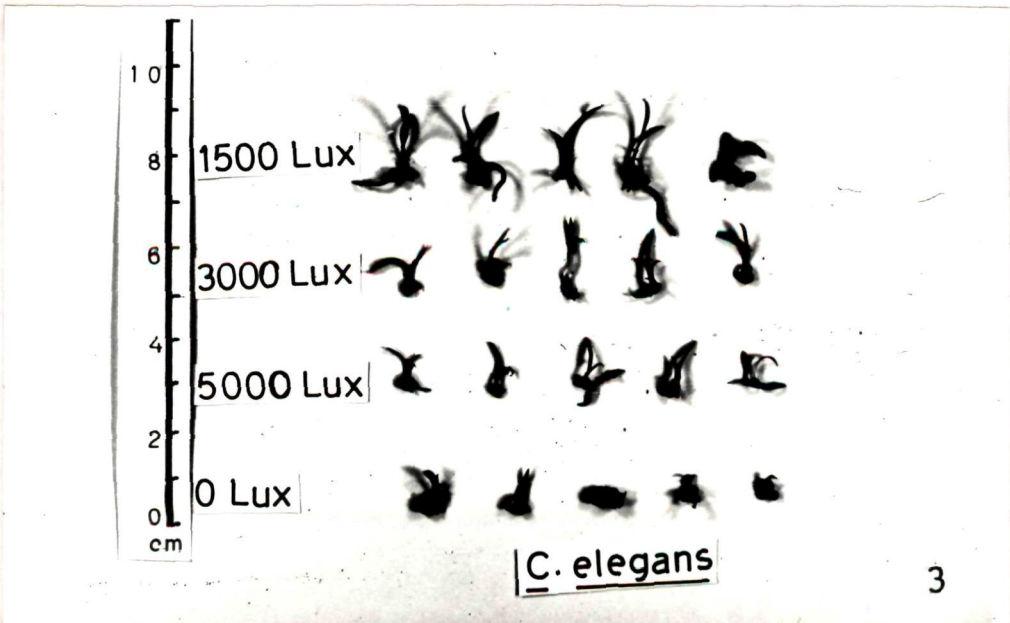
PLATE 7


1 2
1 0
8
6
4
2
0
cm
1500 Lux
3000 Lux
5000 Lux
0 Lux
C. giganteum

1


1 2
1 0
8
6
4
2
0
cm
1500 Lux
3000 Lux
5000 Lux
0 Lux
T. alba

2


1 0
8
6
4
2
0
cm
1500 Lux
3000 Lux
5000 Lux
0 Lux
C. elegans

3

Statistically, significant variations were obtained in relation to the average area of seedlings/plantlets at 1% level between culture periods in all the species and at 5% level between various light intensities in case of C. elegans and C. giganteum. However, variations were not significant in case of T. alba. The development of leaf primordia/leaves also varied with different light intensities (Table 8). The light intensities of 1500 lux and 3000 lux supported the maximum number and area of leaf primordia/leaves in C.elegans and C. giganteum, T. alba respectively. On the other hand, minimum number and area were noticed in the dark in all the cases (Table 8).

The rhizoids/roots were best formed under 1500 lux light intensity in C. elegans and C. giganteum whereas, in T. alba both number and area of rhizoids/roots were highest at 3000 lux light (Table 9). However, higher light intensities and dark conditions were inhibitory for rhizoids/roots development in all the species (Table 9).

b) Effect of light quality

The seed germination and seedling development were variously affected by different light qualities (Fig. 9 and 10, Table 10, Plate 8). The maximum seed germination was noticed in the green and white light in C. elegans and C. giganteum, respectively. Seed germination on the other

Fig. 9 : Seed germination (%) of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, under light of different qualities.

Fig.9

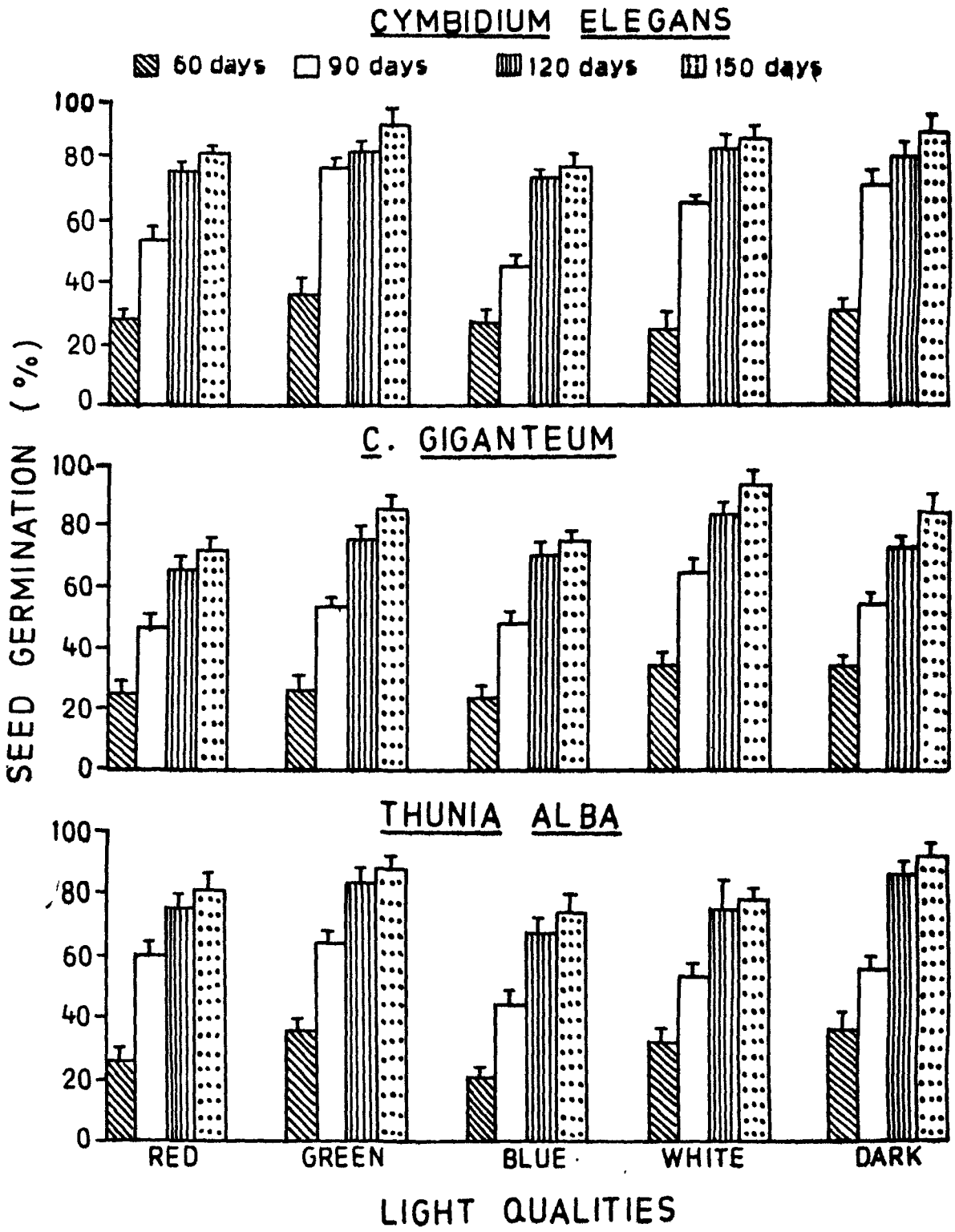

CYMBIDIUM ELEGANS
60 days
90 days
120 days
150 days
100
80
60
40
20
0
C. GIGANTEUM
THUNIA ALBA
SEED GERMINATION (%)
RED
GREEN
BLUE
WHITE
DARK
LIGHT QUALITIES

Fig. 10 : Seedling growth index of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, under light of different qualities.

Fig.10

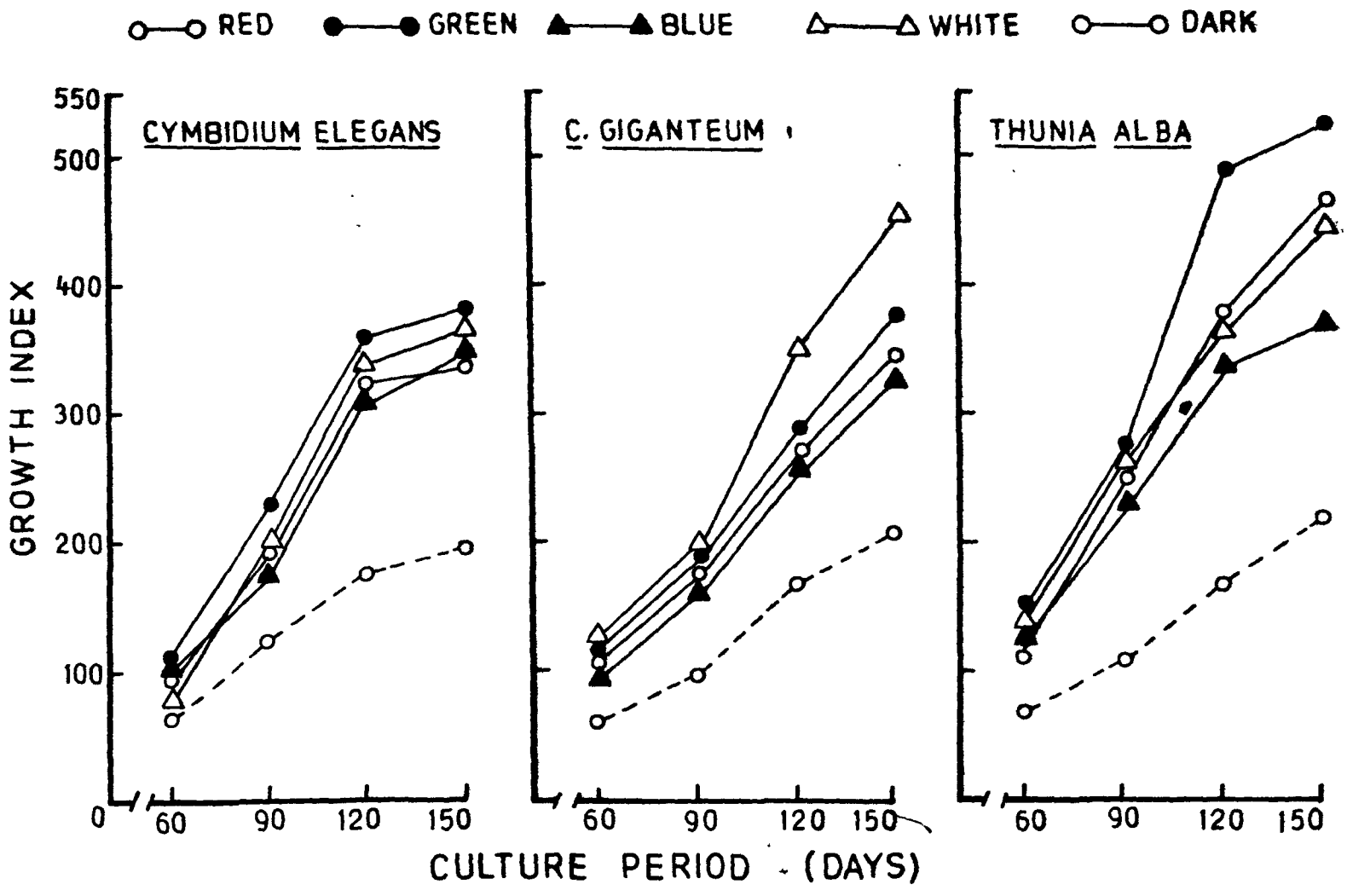

RED
GREEN
BLUE
WHITE
DARK
CYMBIDIUM ELEGANS
C. GIGANTEUM
THUNIA ALBA
GROWTH INDEX
0
100
200
300
400
500
550
60
90
120
150
CULTURE PERIOD (DAYS)

Table 10 : Effect of light qualities on the growth of seedlings/ plantlets of orchids in asymbiotic conditions

Orchid species	Culture period (days)	Average area of seedlings/plantlets (mm^2)				
		red	green	blue	white	dark
C. elegans	60	0.11	0.23	0.03	0.13	0.03
	90	0.44	1.00	0.11	0.65	0.09
	120	3.13	5.11	1.95	3.76	0.32
	150	3.77	7.68	2.40	4.69	2.25
C. giganteum	60	0.17	0.69	0.11	0.84	0.10
	90	1.98	4.79	0.46	6.19	0.41
	120	11.00	15.05	6.63	20.46	3.11
	150	13.48	17.04	7.59	22.81	4.61
T. alba	60	0.10	0.18	0.08	0.03	0.01
	90	0.37	1.17	0.16	0.19	0.06
	120	13.27	15.53	12.50	12.95	4.10
	150	14.56	18.49	13.38	13.79	7.48

Table 11 : Effect of light qualities on the production of leaf primordia/leaves of orchids in asymbiotic conditions

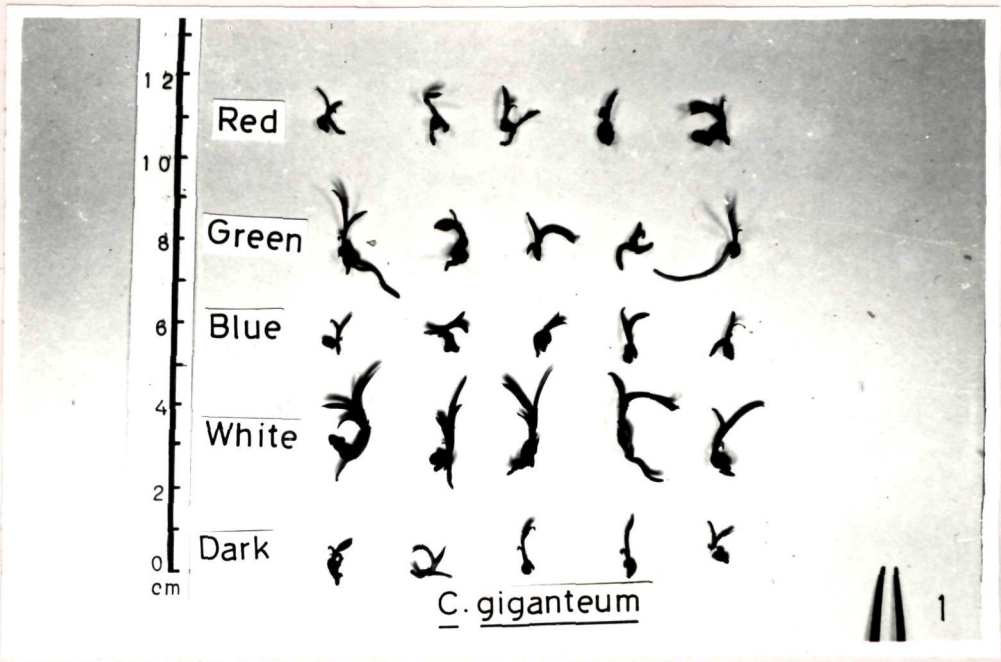
Orchid species	Culture period (days)	Average number and area of leaf primordia/leaves (mm^2)									
		red		green		blue		white		dark	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	0.92	0.02	1.19	0.05	-	-	1.02	0.03	-	-
	90	1.26	0.07	1.74	0.16	0.92	0.02	1.20	0.08	0.58	0.008
	120	1.48	0.19	2.33	1.66	1.15	0.59	1.89	1.03	0.79	0.01
	150	1.75	0.76	2.66	3.60	1.32	0.67	2.03	1.17	1.23	0.20
C. giganteum	60	-	-	0.49	0.05	-	-	0.79	0.10	-	-
	90	0.59	0.13	1.17	0.61	0.82	0.07	1.66	0.98	0.36	0.04
	120	1.72	1.03	1.61	1.45	1.67	0.96	2.35	2.24	1.00	0.33
	150	1.81	1.17	1.78	1.69	1.76	1.07	2.57	2.88	1.28	0.68
T. alba	60	0.94	0.04	1.11	0.03	0.76	0.008	-	-	-	-
	90	1.40	0.28	1.71	0.56	1.16	0.04	0.92	0.03	0.55	0.003
	120	1.89	0.36	2.45	2.33	1.79	1.33	1.41	0.88	1.23	0.32
	150	2.20	1.60	2.73	2.81	2.09	1.48	1.47	1.03	1.38	0.39

Table 12 : Effect of light qualities on the production of rhizoids/roots of orchids in asymbiotic conditions

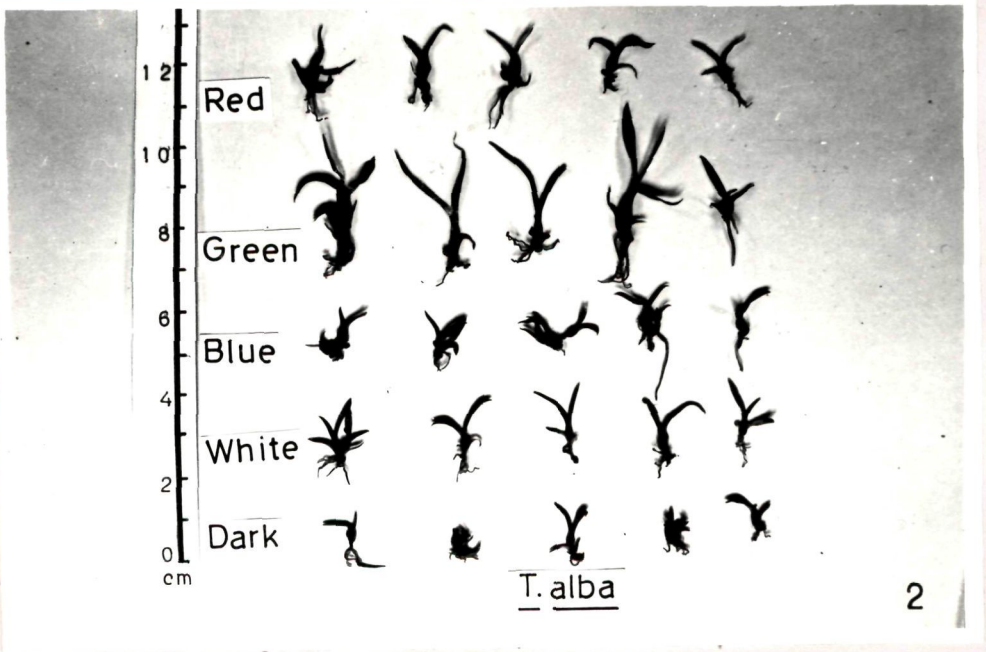
Orchid species	Culture period (days)	Average number of area of rhizoids/roots (mm^2)									
		red		green		blue		white		dark	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	5.20	0.002	5.18	0.002	2.36	0.001	3.69	0.001	3.76	0.001
	90	5.65	0.05	10.36	0.005	4.80	0.002	7.17	0.004	4.87	0.003
	120	7.51	0.21	11.85	0.26	6.51	0.13	7.62	0.20	9.28	0.004
	150	9.26	0.23	12.31	0.31	7.50	0.18	9.70	0.27	10.37	0.10
C. giganteum	60	2.12	0.001	5.46	0.001	5.26	0.0014	6.05	0.006	4.52	0.0012
	90	6.52	0.003	10.61	0.18	12.39	0.005	13.50	0.25	6.66	0.0015
	120	7.70	0.45	11.23	0.94	12.56	0.15	16.26	1.15	12.34	0.04
	150	8.96	0.52	12.50	1.02	13.40	0.20	17.57	1.41	14.89	0.14
T. alba	60	1.05	0.0018	1.10	0.003	0.85	0.0019	0.52	0.0004	0.72	0.0014
	90	1.67	0.006	1.77	0.23	1.33	0.05	1.30	0.002	1.37	0.003
	120	2.61	0.71	2.75	0.73	2.24	0.42	2.03	0.37	1.71	0.16
	150	2.85	0.93	3.07	1.04	2.87	0.62	2.20	0.53	1.82	0.19

Plate 8 : Seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), under light of different qualities.

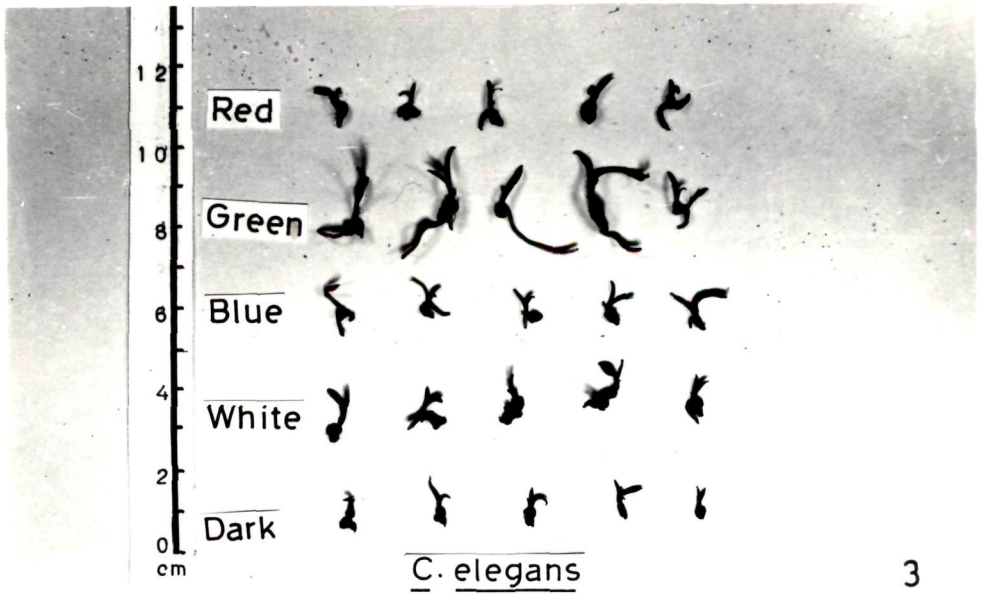
PLATE-8



C. giganteum 1



T. alba 2



C. elegans 3

hand, was enhanced in the dark conditions in case of T. alba (Fig. 9). The lowest percentage of seed germination was obtained in the blue and red light regimes in C. elegans, T. alba, and C. giganteum, respectively (Fig. 9).

The data analysis revealed significant variation at 1% level between different light qualities and culture period in relation to the seed germination in all the three species.

The highest growth index was noticed under the green light in C. elegans and T. alba, but C. giganteum exhibited better growth index in white light than the other light qualities. The lowest growth index was recorded in the dark condition in all the species (Fig. 10). The growth index was significantly different at 1% level between light qualities and culture periods in all the species studied. The average area of seedlings/plantlets was also enhanced in the green light in case of C. elegans and T. alba whereas, C. giganteum exhibited maximum area of seedlings/plantlets in white light (Table 10). The minimum area of seedlings resulted in the dark conditions in all the cases. The average area of seedlings/plantlets was significantly different at 1% level between different culture periods but the difference in the area of seedlings/plantlets between the light qualities was found to be insignificant in all the

Fig. 11 : Seed germination (%) of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, under different photoperiods.

Fig.11

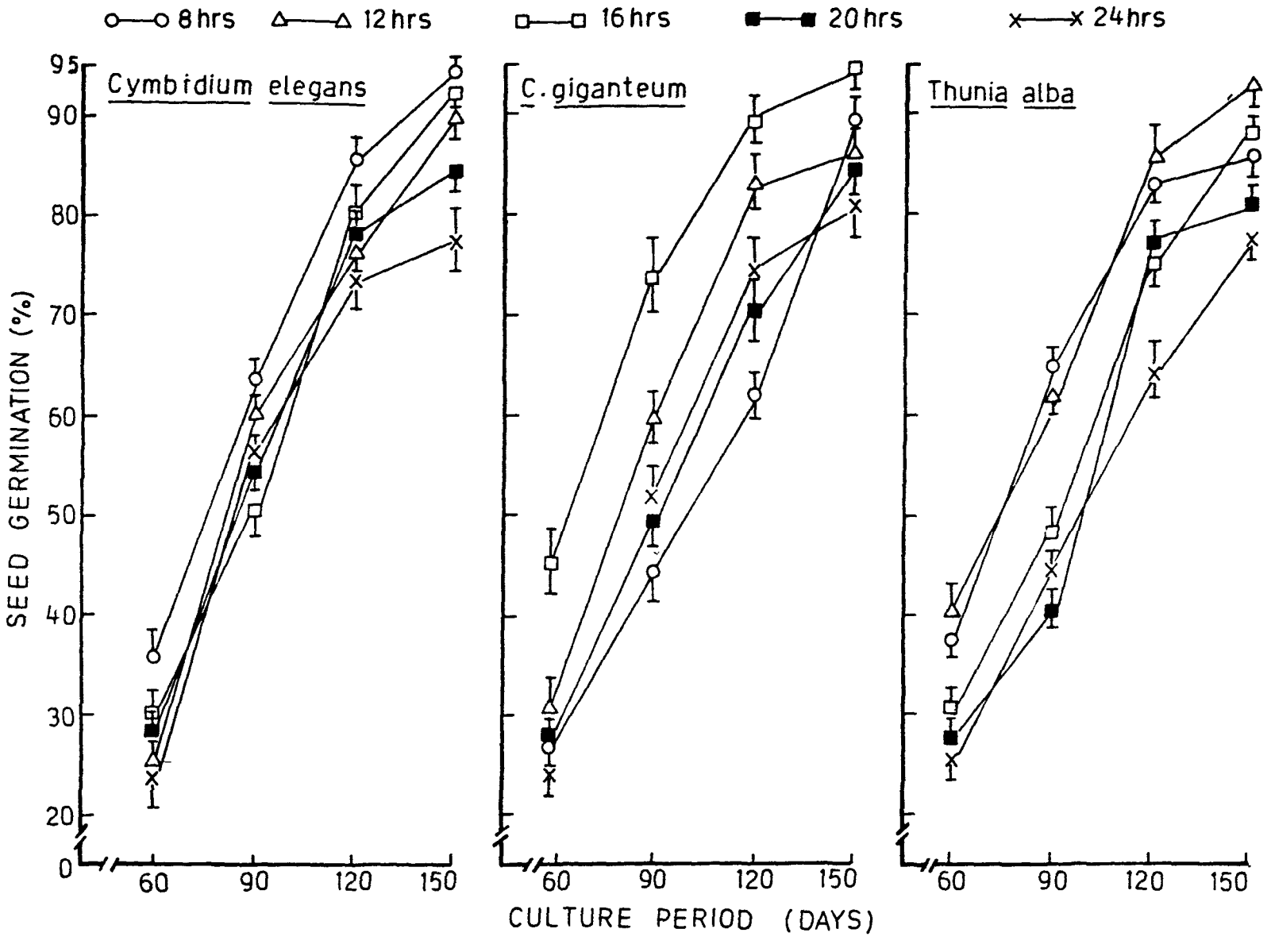

8hrs
12hrs
16hrs
20hrs
24hrs
Cymbidium elegans
C. giganteum
Thunia alba
SEED GERMINATION (%)
0
20
30
40
50
60
70
80
90
95
60
90
120
150
CULTURE PERIOD (DAYS)

Fig. 12 : Seedling growth index of *Cymbidium elegans*, *C. giganteum* and *Thunia alba*, under different photoperiods.

Fig 12

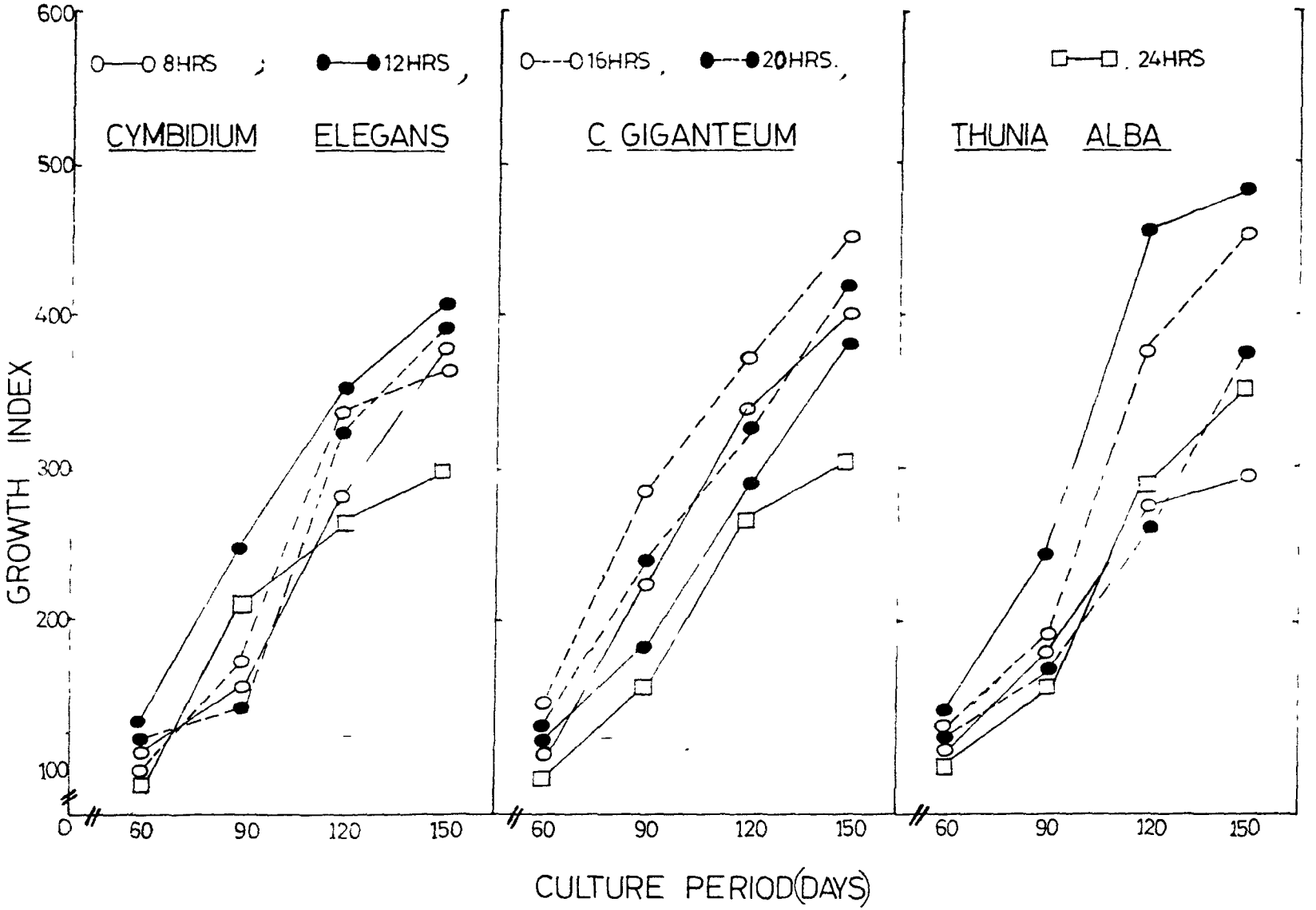

8HRS
12HRS
16HRS
20HRS.
24HRS
CYMBIDIUM ELEGANS
C GIGANTEUM
THUNIA ALBA
GROWTH INDEX
600
500
400
300
200
100
0
60
90
120
150
CULTURE PERIOD(DAYS)

Table 13 : Effect of photoperiods on the growth of seedlings/ plantlets of orchids in asymbiotic conditions

Orchid species	Culture period (days)	Average area of seedlings/plantlets (mm^2)				
		8 hr	12 hr	16 hr	20 hr	24 hr
C. elegans	60	0.08	0.17	0.13	0.09	0.05
	90	0.45	2.00	0.68	0.40	0.34
	120	2.87	6.75	4.75	3.83	1.51
	150	3.41	8.76	5.76	5.40	3.24
C. giganteum	60	0.32	0.67	0.90	0.59	0.11
	90	1.49	3.84	6.54	2.46	0.43
	120	7.56	11.31	24.44	17.05	4.09
	150	9.26	15.13	29.43	20.23	11.37
T. alba	60	0.09	0.19	0.09	0.03	0.01
	90	0.20	1.06	0.21	0.20	0.06
	120	2.62	7.26	3.18	3.41	1.29
	150	13.75	18.52	15.56	14.54	11.13

Table 14 : Effect of photoperiods on the production of leaf primordia/leaves of orchids in asymbiotic conditions

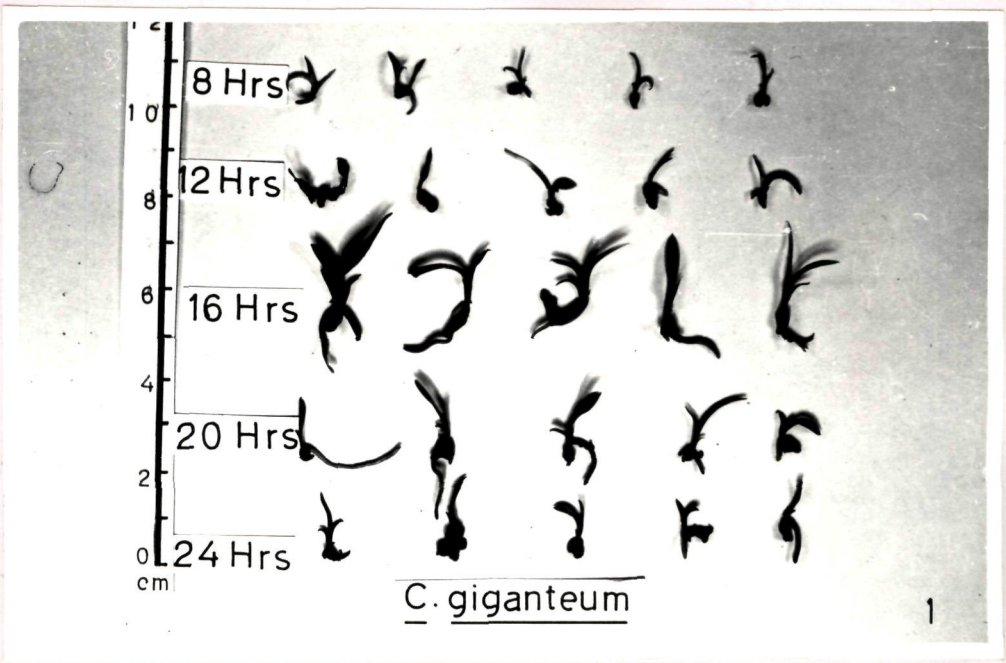
Orchid species	Culture period (days)	Average number and area of leaf primordia/leaves (mm^2)									
		8 hr		12 hr		16 hr		20 hr		24 hr	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	1.22	0.05	1.42	0.09	1.36	0.07	1.12	0.06	1.02	0.03
	90	1.58	0.16	1.95	0.41	1.73	0.27	1.52	0.17	1.19	0.05
	120	2.07	1.29	2.86	2.46	2.16	1.25	1.95	0.82	1.33	0.10
	150	2.41	2.06	3.28	3.86	2.77	2.04	2.14	1.23	1.45	0.44
C. giganteum	60	0.63	0.02	0.77	0.04	0.91	0.12	0.72	0.06	-	-
	90	0.77	0.10	1.06	0.24	1.26	0.30	0.90	0.14	0.72	0.05
	120	1.40	0.71	1.90	1.06	1.99	1.49	1.65	1.43	1.06	0.24
	150	1.63	1.11	2.35	1.78	2.41	2.53	2.32	1.96	1.69	1.24
T. alba	60	0.95	0.03	1.13	0.04	0.89	0.02	-	-	-	-
	90	1.31	0.16	1.41	0.19	1.17	0.09	1.00	0.07	0.65	0.007
	120	1.93	1.10	2.37	2.34	1.75	1.12	1.28	0.98	0.88	0.04
	150	2.25	1.89	2.76	2.93	2.24	1.92	1.79	1.35	1.37	0.44

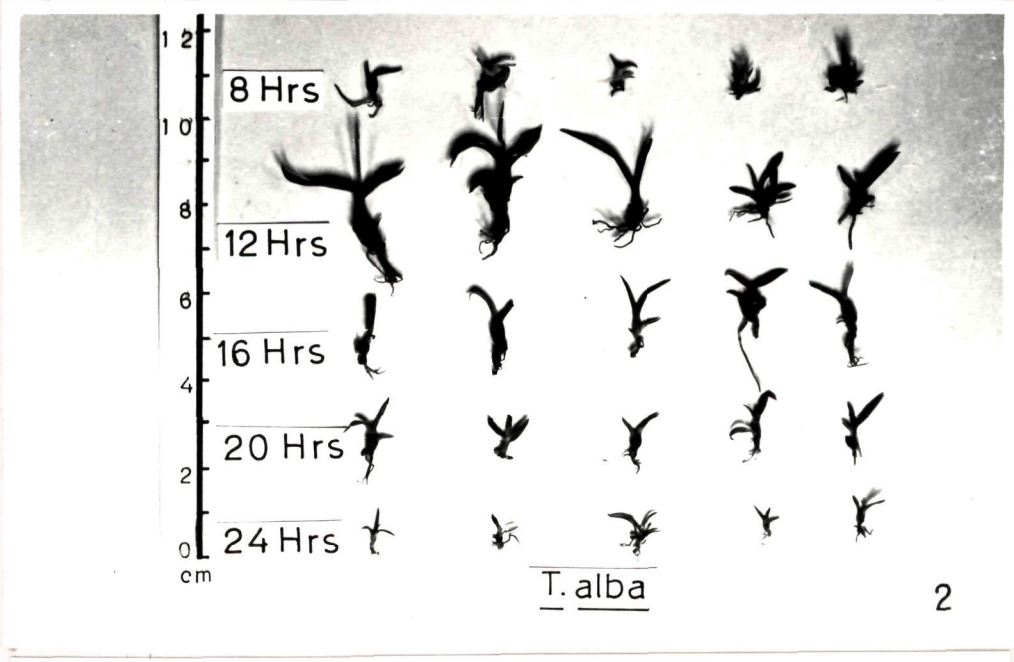
Table 15 : Effect of photoperiods on the production of rhizoids/roots of orchids in asymbiotic conditions

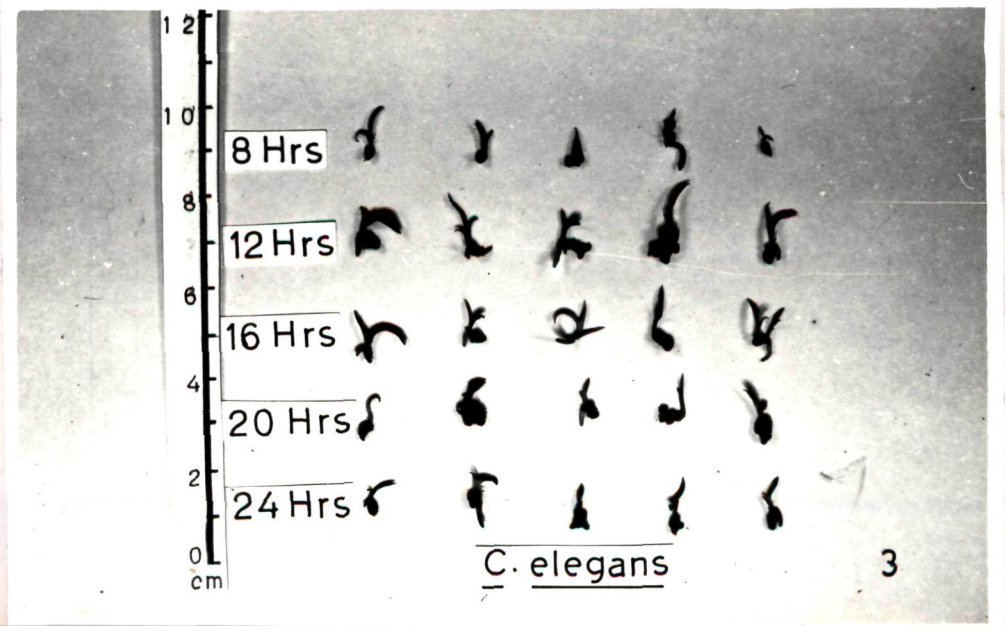
Orchid species	Culture period (days)	Average number and area of rhizoids/roots (mm^2)									
		8 hr		12 hr		16 hr		20 hr		24 hr	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	60	5.26	0.0002	5.33	0.001	5.41	0.002	5.47	0.001	4.56	0.0007
	90	8.49	0.004	8.68	0.05	10.98	0.01	9.42	0.003	6.26	0.002
	120	9.15	0.21	9.13	0.25	11.26	0.37	10.15	0.30	6.88	0.03
	150	10.18	0.37	11.20	0.40	13.12	0.49	11.26	0.48	8.04	0.29
C. giganteum	60	5.34	0.0009	3.75	0.001	6.60	0.001	5.20	0.0008	3.08	0.0005
	90	11.06	0.004	7.60	0.16	9.98	0.04	9.61	0.002	8.03	0.003
	120	12.96	0.36	8.27	0.52	10.25	0.96	9.85	0.54	10.61	0.05
	150	13.45	0.48	10.45	0.66	15.06	1.46	10.80	0.87	11.88	0.30
T. alba	60	0.31	0.0006	1.00	0.002	0.77	0.002	0.46	0.0004	0.65	0.0003
	90	1.00	0.002	1.80	0.29	1.38	0.01	1.29	0.006	1.27	0.003
	120	1.69	0.16	2.73	0.87	1.96	0.22	1.71	0.17	1.73	0.03
	150	1.89	0.31	3.53	1.38	3.07	0.63	2.53	0.27	1.87	0.15

Plate 9 : Seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), under different photoperiods.

PLATE 9


8 Hrs
12 Hrs
16 Hrs
20 Hrs
24 Hrs
cm
C. giganteum
1


8 Hrs
12 Hrs
16 Hrs
20 Hrs
24 Hrs
cm
T. alba
2


8 Hrs
12 Hrs
16 Hrs
20 Hrs
24 Hrs
cm
C. elegans
3

species except in C. elegans.

The formation and development of leaves and roots also varied under different light qualities (Tables 11 and 12). The highest number and area of leaf and root occurred under green light in C. elegans and T. alba while, white light induced better root and leaf formation in C. giganteum (Tables 11 and 12).

c) Effect of photoperiod

The seed germination and seedlings growth varied under different photoperiods (Figs. 11 and 12, Table 13, Plate 9). Seed germination was uniformly at its lowest percentage in 24 hr photoperiod. It was significantly higher between 8-16 hr photoperiod (Fig. 11). The maximum seed germination was recorded under 8, 12 and 16 hr photoperiods in C. elegans, T. alba and C. giganteum, respectively (Fig. 11). The highest and lowest growth index of seedling development were noticed under 12 and 8 hr photoperiods respectively in T. alba. C. elegans and C. giganteum exhibited higher growth index at 12 and 16 hr photoperiod, respectively. On the other hand the lowest growth index was recorded under 24 hr photoperiod in both the species (Fig. 12). Significant variations were obtained between different photoperiods and culture periods in relation to the seed germination and growth index at 1% level and 5% level, respectively.

The average area of seedlings/plantlets was maximum under 12 hr photoperiod in C. elegans and T. alba whereas, in C. giganteum it was highest at 16 hr photoperiod. The seedling growth was inhibited under 24 hr photoperiod in all the species (Table 13, Plate 9). Statistically insignificant variations were observed in relation to the average area of seedlings/plantlets between different photoperiods but variations were significant at 1% level between culture periods.

The development of leaf primordia/leaves also varied at different photoperiods. The maximum number and area of leaf primordia/leaves were obtained under 12 hr photoperiod in C. elegans and T. alba while, leaf production was highest at 16 hr photoperiod in case of C. giganteum (Table 14). The highest average number and area of rhizoids/roots occurred under 16 hr photoperiod in both the Cymbidium species but in T. alba these were highest at 12 hr photoperiod. The 24 hr photoperiod resulted in minimum number and area of rhizoids/roots in all the species (Table 15).

II. Effect of Temperature, pH and Light on Symbiotic Seed Germination and subsequent Seedling Growth of Orchids

1) Effect of temperature

The symbiotic germination of seeds and seedling

Fig. 13 : Symbiotic seed germination (%) of Cymbidium elegans, at different temperatures.

Fig.13

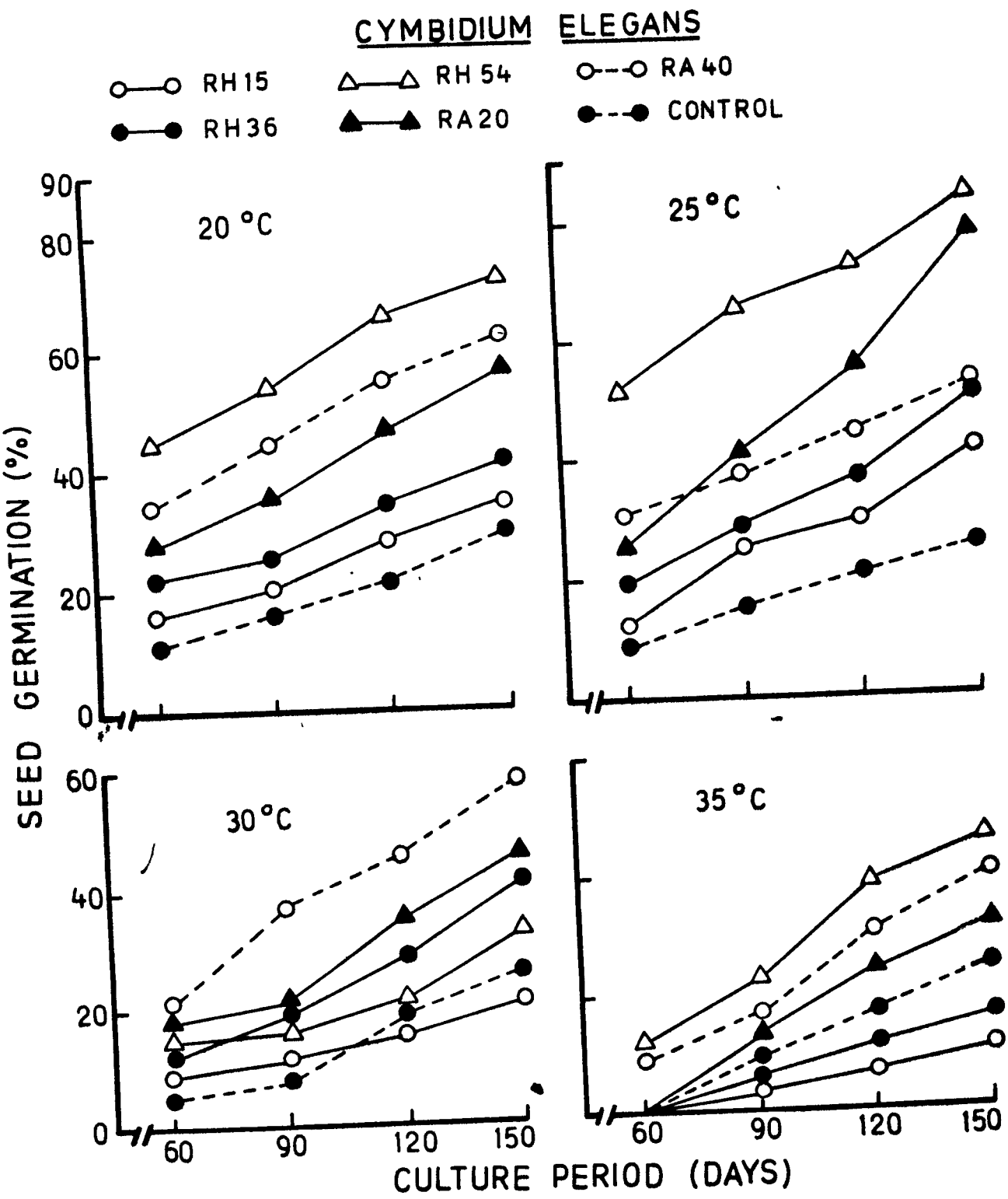

CYMBIDIUM ELEGANS
RH 15
RH 54
RA 40
RH 36
RA 20
CONTROL
20 °C
25 °C
30 °C
35 °C
SEED GERMINATION (%)
CULTURE PERIOD (DAYS)
0
20
40
60
80
90
120
150

Fig. 14 : Symbiotic seed germination (%) of Cymbidium giganteum, at different temperatures.

Fig 14

CYMBIDIUM GIGANTEUM

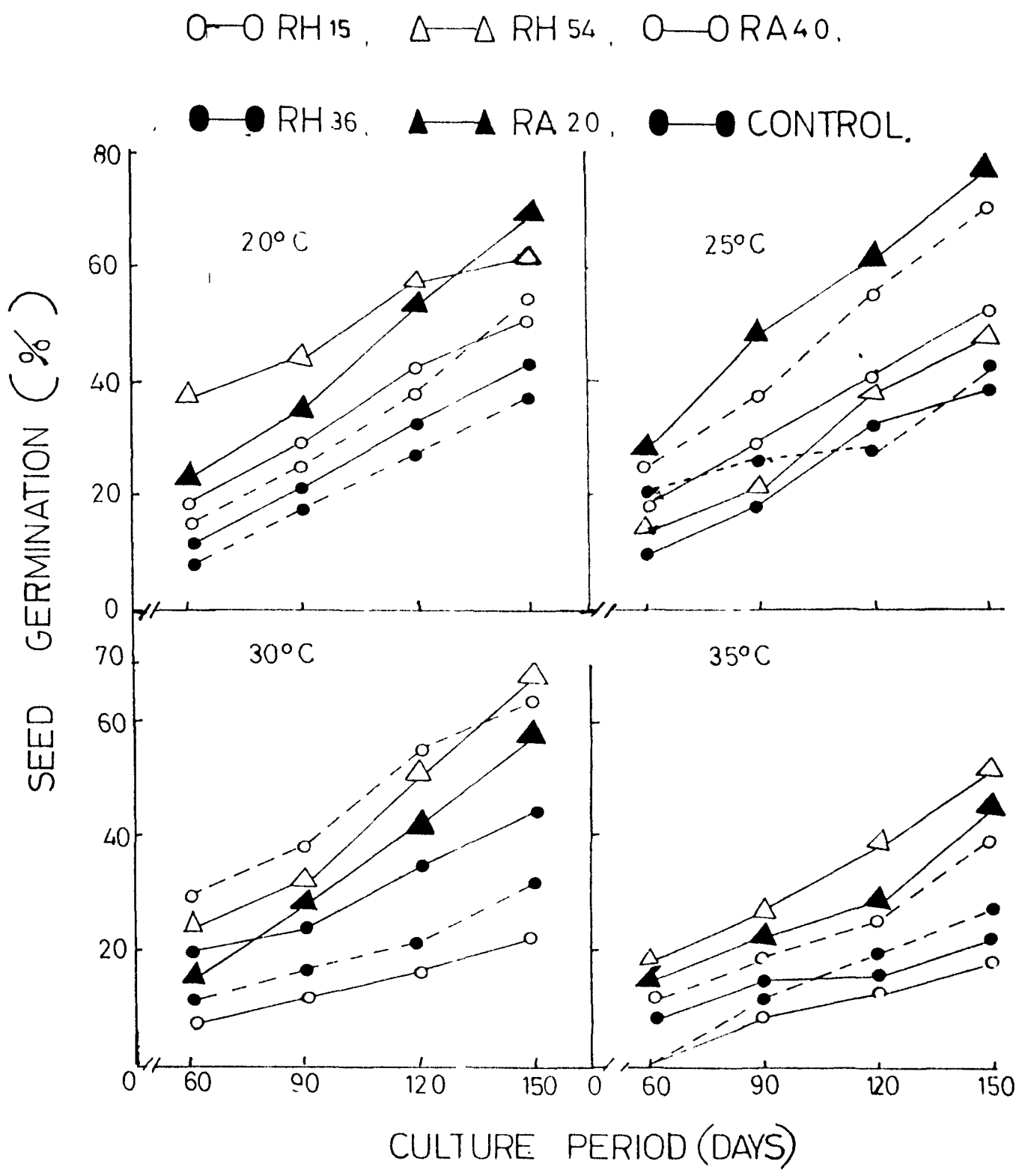


Fig. 15 : Symbiotic seed germination (%) of *Thunia alba*, at different temperatures.

Fig.15

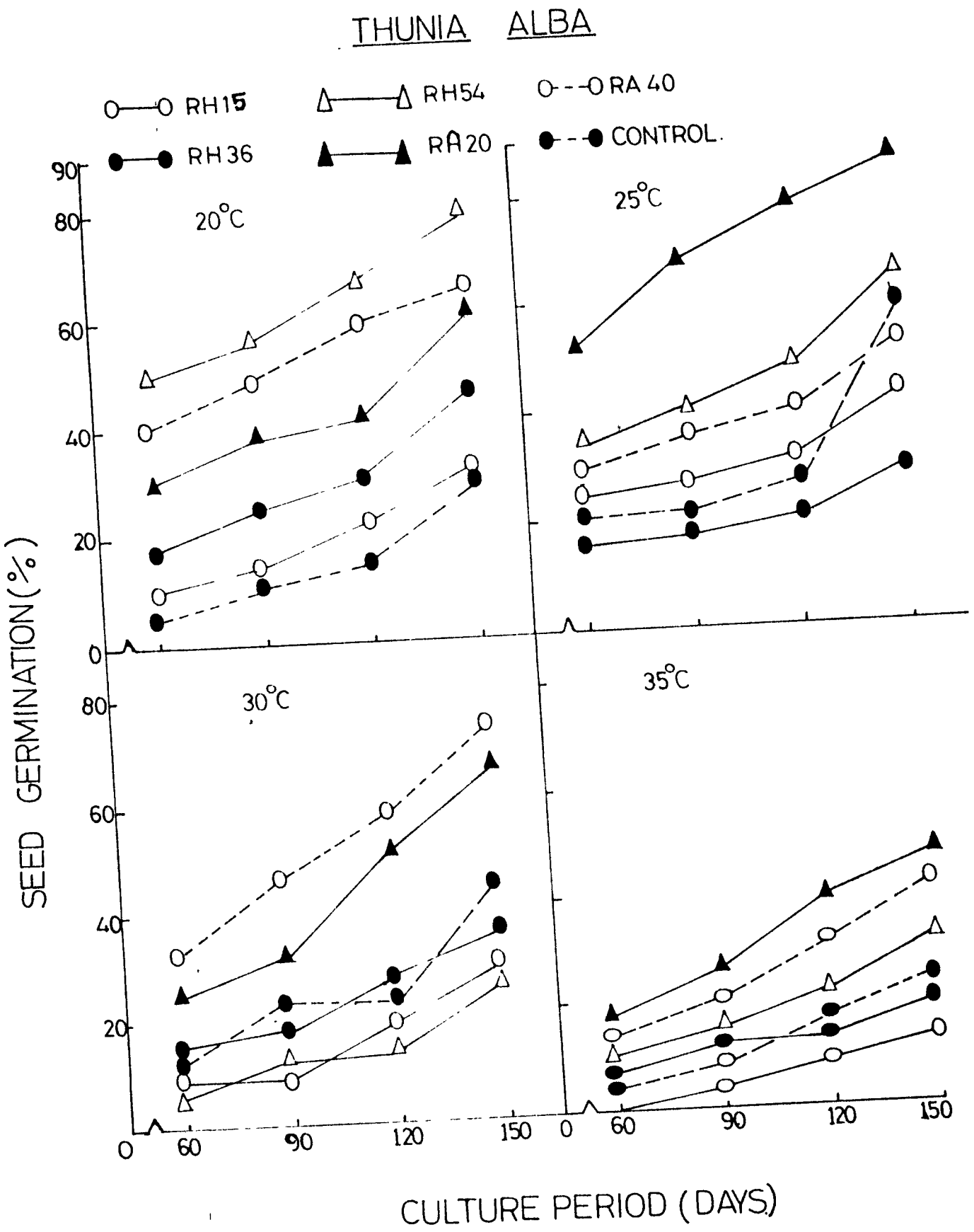

THUNIA ALBA
RH15
RH54
RA 40
RH36
RA20
CONTROL
20°C
25°C
30°C
35°C
SEED GERMINATION (%)
CULTURE PERIOD (DAYS)
0
20
40
60
80
90
120
150

Fig. 16 : Symbiotic seedling growth index of *Cymbidium elegans*, at different temperatures.

Fig.16

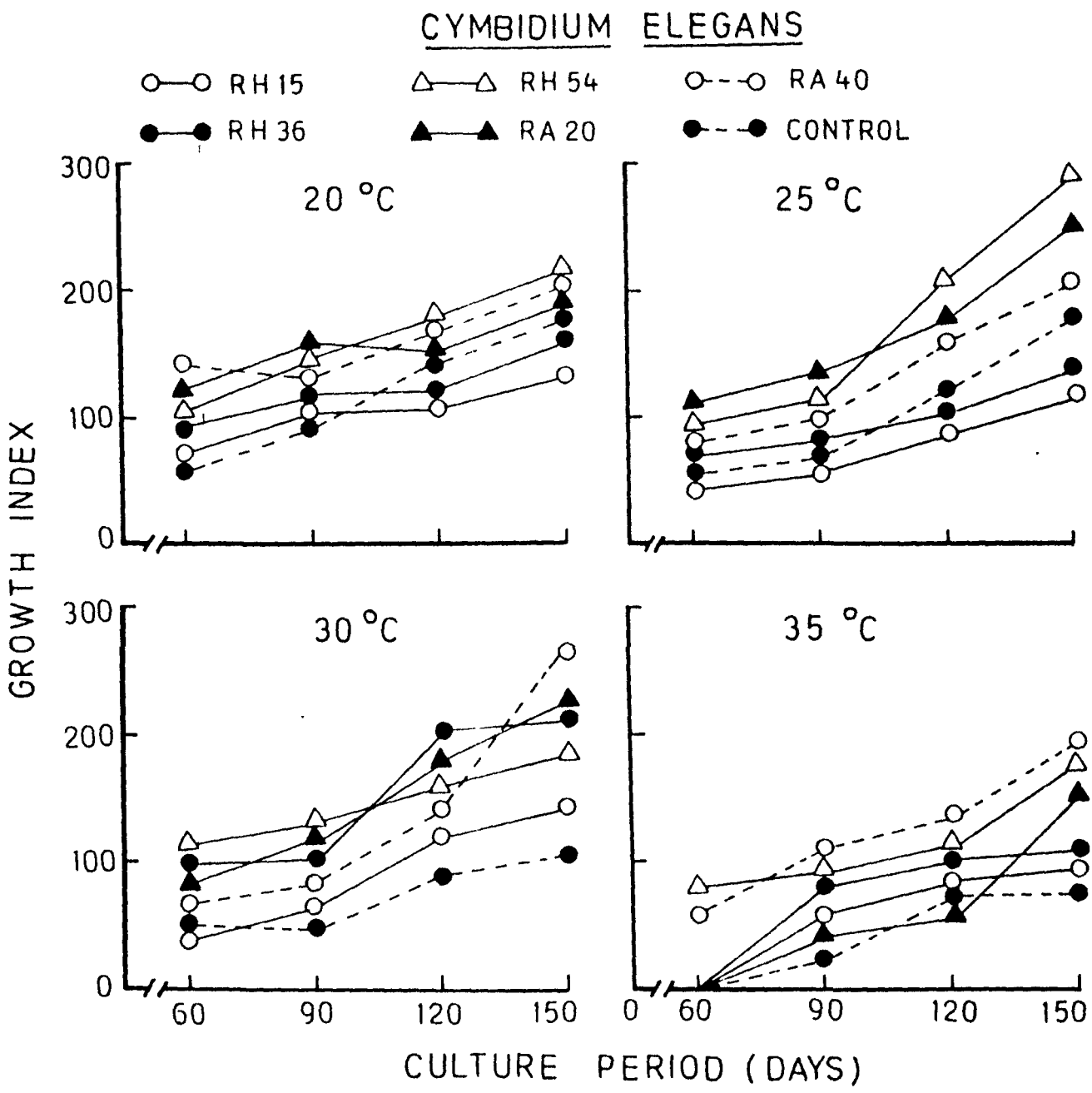

CYMBIDIUM ELEGANS
RH 15
RH 36
RH 54
RA 20
RA 40
CONTROL
20 °C
25 °C
30 °C
35 °C
GROWTH INDEX
300
200
100
0
60
90
120
150
CULTURE PERIOD (DAYS)

Fig. 17 : Symbiotic seedling growth index of *Cymbidium giganteum*, at different temperatures.

Fig.17

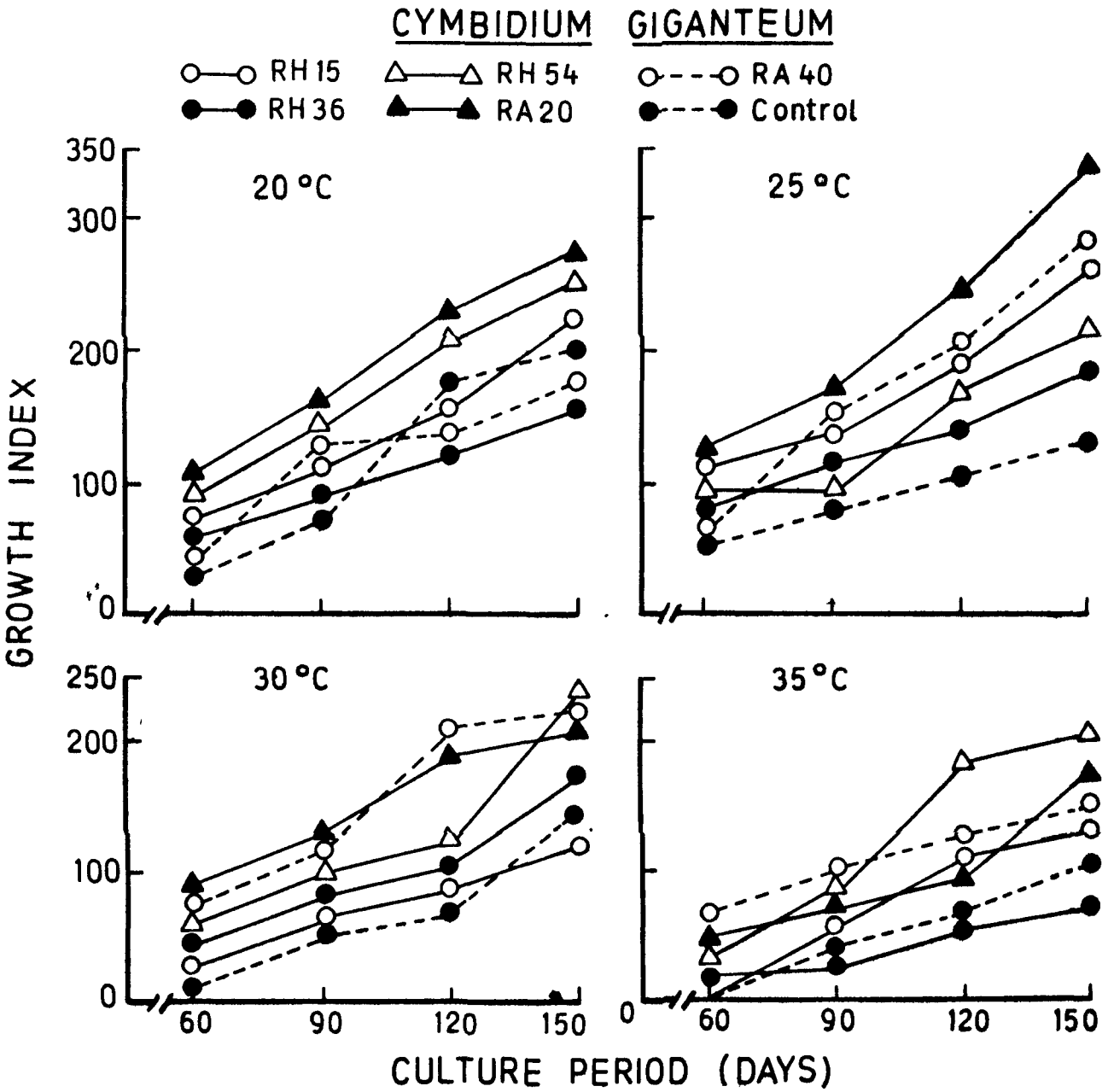
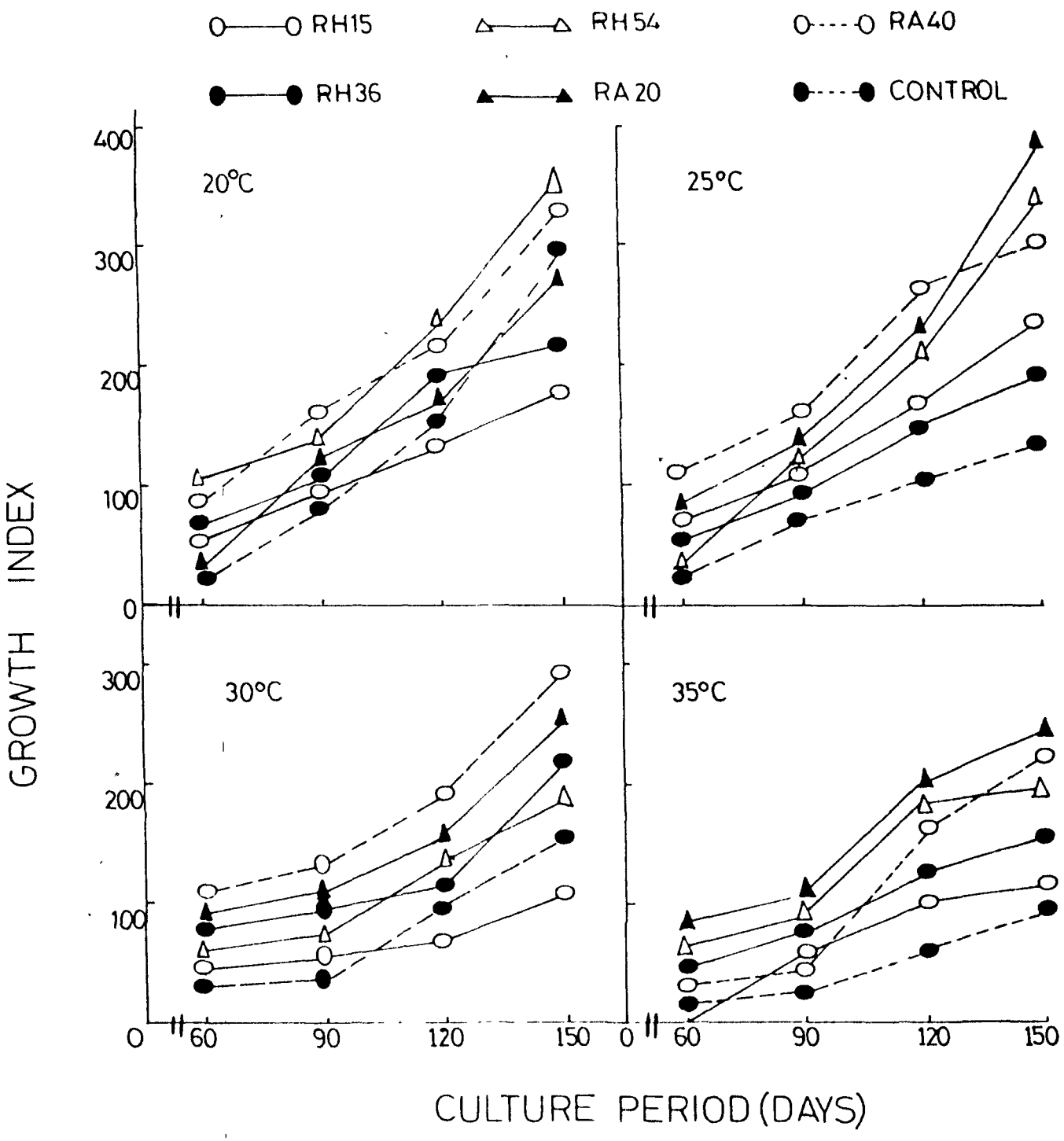

CYMBIDIUM GIGANTEUM
RH 15
RH 36
RH 54
RA 20
RA 40
Control
20 °C
25 °C
30 °C
35 °C
GROWTH INDEX
CULTURE PERIOD (DAYS)
0
100
200
300
350
250
60
90
120
150

Fig. 18 : Symbiotic seedling growth index of *Thunia alba*, at different temperatures.

Fig 18

THUNIA ALBA



development varied with respect to the temperatures (Plate 10, Figs. 13-15). The maximum seed germination was noticed at 25°C with RH15, RH36, RH54 and RA20. However, with RA40 comparatively higher percentage of seed germination resulted at 20°C. The seed germination was the lowest with all the fungal endophytes at 35°C in C. elegans (Fig. 13). Cymbidium giganteum showed the higher seed germination with RH36, RH54 and RH15, RA20, RA40 at 30° and 25°C, respectively. At 35°C the seed germination was inhibited in all the cases (Fig. 14). The seed germination in T. alba was higher at 20°C using RH36 and RH54 isolates but with RA20 and RA40 better germination was recorded at 25 and 30°C, respectively (Fig. 15). Seed germination was significantly different at 1% level between different temperatures and at 5% level between different fungal isolates in all the species.

The maximum growth index in C. elegans was obtained using RH54 followed by RA20 at 25°C. On the other hand, growth index was enhanced at 30°C using RH15, RH36 and RA40 isolates (Fig. 16). In case of C. giganteum, the growth index was significantly higher at 25°C with RH15, RH36, RA20 and RA40 isolates than the other temperatures whereas, growth index increased at 20°C using RH54 endophyte (Fig. 17). Significantly higher growth index was observed using RH36, RH54 and RA40 at 20°C and with RH15 and RA20 endophytes at 25°C in T. alba (Fig. 18). Growth index, however, decreased

Table 16 : Effect of temperature on the growth of seedlings/ plantlets of orchids in symbiotic conditions

Orchid species	Fungal isolates	Average area of seedlings/plantlets (mm^2)			
		20°C	25°C	30°C	35°C
	RH 15	1.21	1.25	1.02	0.82
	RH 36	1.73	2.54	1.15	0.96
C. elegans	RH 54	4.56	5.35	1.33	1.46
	RA 20	2.12	4.19	1.45	1.55
	RA 40	3.06	3.55	2.49	1.67
	Control	1.15	1.36	1.06	0.75
	RH 15	5.16	8.96	2.64	1.17
	RH 36	3.23	4.13	4.76	1.02
C. giganteum	RH 54	8.79	9.96	10.12	8.11
	RA 20	12.26	16.75	7.69	4.92
	RA 40	5.45	18.45	9.55	3.15
	Control	7.67	10.54	4.99	2.45
	RH 15	4.90	4.98	3.60	2.13
	RH 36	2.67	3.99	7.95	3.76
T. alba	RH 54	11.67	12.26	6.22	5.15
	RA 20	9.47	10.05	8.45	10.96
	RA 40	10.82	11.03	10.95	7.63
	Control	6.32	8.97	7.92	2.32

Table 17 : Effect of temperature on the production of leaf primordia/leaves of orchids in symbiotic conditions, after 150 days

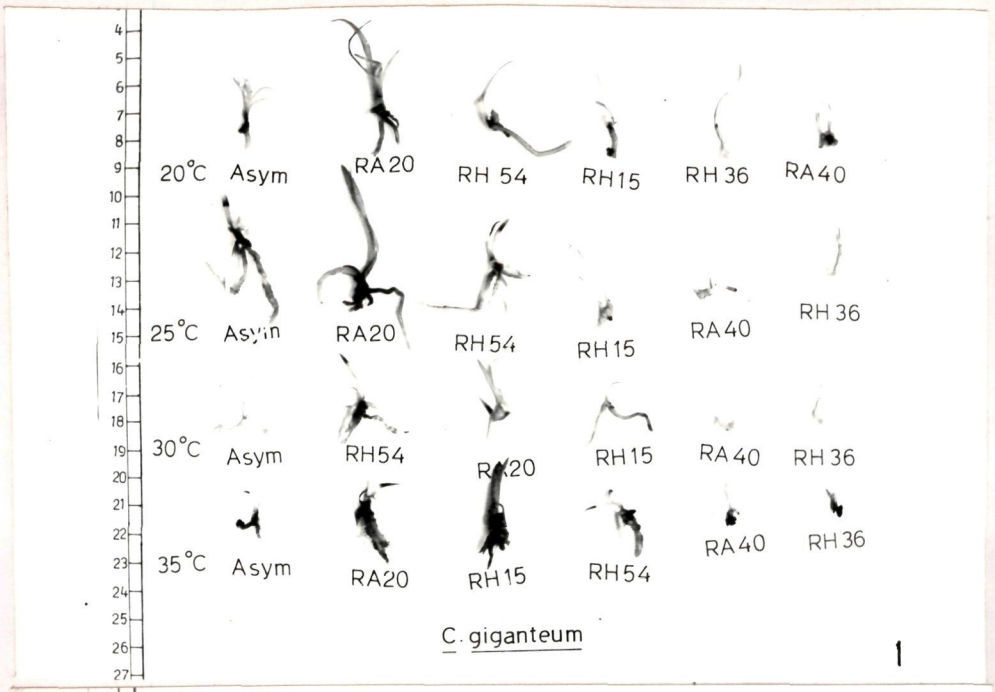
Orchid species	Fungal isolates	Average number and area of leaf primordia/leaves (cm^2)							
		20°C		25°C		30°C		35°C	
		No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	1.87	1.71	2.11	0.89	1.59	0.45	0.99	0.26
	RH 36	2.15	1.35	2.17	1.02	1.46	0.61	1.42	0.27
	RH 54	1.96	1.42	2.25	1.11	1.63	0.71	1.22	0.31
	RA 20	2.11	1.56	2.31	1.26	1.79	0.69	1.36	0.35
	RA 40	2.32	1.85	2.67	1.45	1.65	0.57	1.59	0.41
	Control	1.57	1.38	1.47	1.44	1.25	0.39	1.12	0.25
C. giganteum	RH 15	1.89	1.35	2.17	1.66	1.86	1.62	1.03	0.54
	RH 36	1.95	1.49	2.32	1.57	1.77	1.71	1.21	0.67
	RH 54	1.79	1.76	2.41	1.49	2.14	1.96	1.29	0.79
	RA 20	2.15	1.85	2.36	1.84	2.12	1.42	1.51	0.59
	RA 40	2.67	1.30	2.54	1.96	2.00	1.35	1.64	0.66
	Control	1.45	1.13	1.69	1.36	1.56	1.50	1.26	0.25
T. alba	RH 15	1.79	1.36	2.00	1.96	1.06	0.81	0.75	0.45
	RH 36	1.56	1.47	2.21	2.15	2.15	1.21	0.96	0.39
	RH 54	1.42	1.62	2.15	2.24	2.19	1.23	1.22	0.68
	RA 20	1.63	1.51	1.98	1.76	1.76	1.45	1.26	0.54
	RA 40	1.75	1.95	1.88	1.95	1.45	1.27	1.36	0.75
	Control	1.44	1.40	1.57	1.45	1.30	1.20	0.67	0.39

Table 18 : Effect of temperature on the production of rhizoids/roots of orchids in symbiotic conditions, after 150 days

Orchid species	Fungal isolates	Average number and area of rhizoids/roots (mm^2)							
		20°C		25°C		30°C		35°C	
		No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	10.62	0.12	6.72	0.15	7.65	0.10	5.76	0.004
	RH 36	16.79	0.16	8.65	0.12	8.54	0.12	6.96	0.010
	RH 54	17.15	0.17	9.55	0.28	9.45	0.25	8.45	0.012
	RA 20	15.92	0.26	12.16	0.25	10.11	0.13	10.55	0.12
	RA 40	17.23	0.28	14.44	0.31	11.25	0.25	9.76	0.10
	Control	9.02	0.25	10.55	0.28	7.65	0.19	5.82	0.019
C. giganteum	RH 15	8.96	0.79	12.49	1.02	7.86	0.61	4.15	0.19
	RH 36	9.75	0.89	10.32	1.15	8.25	0.72	5.65	0.26
	RH 54	10.15	1.02	9.62	0.96	9.99	0.81	5.39	0.20
	RA 20	11.49	1.12	11.11	0.79	8.44	0.77	6.72	0.35
	RA 40	10.00	0.62	12.23	1.25	7.50	0.85	6.85	0.41
	Control	7.00	0.47	8.95	0.65	5.56	0.45	3.98	0.33
T. alba	RH 15	1.22	0.17	1.95	0.26	1.85	0.17	1.29	0.11
	RH 36	1.36	0.21	2.02	0.21	2.11	0.22	1.45	0.14
	RH 54	1.55	0.23	2.36	0.24	2.67	0.27	1.65	0.15
	RA 20	1.82	0.27	2.54	0.38	1.57	0.36	1.77	0.26
	RA 40	1.76	0.31	3.17	0.41	1.97	0.45	1.80	0.28
	Control	1.69	0.19	1.80	0.35	1.74	0.26	1.66	0.17

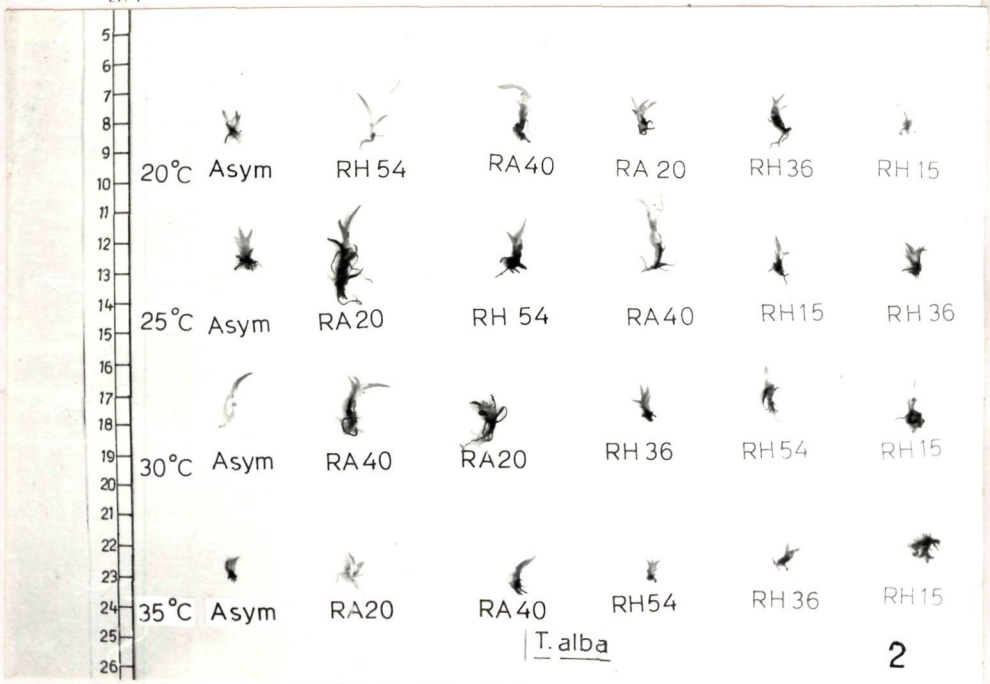
Plate 10 : Symbiotic seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2), and *C. elegans* (Fig. 3), at different temperatures.

PLATE 10



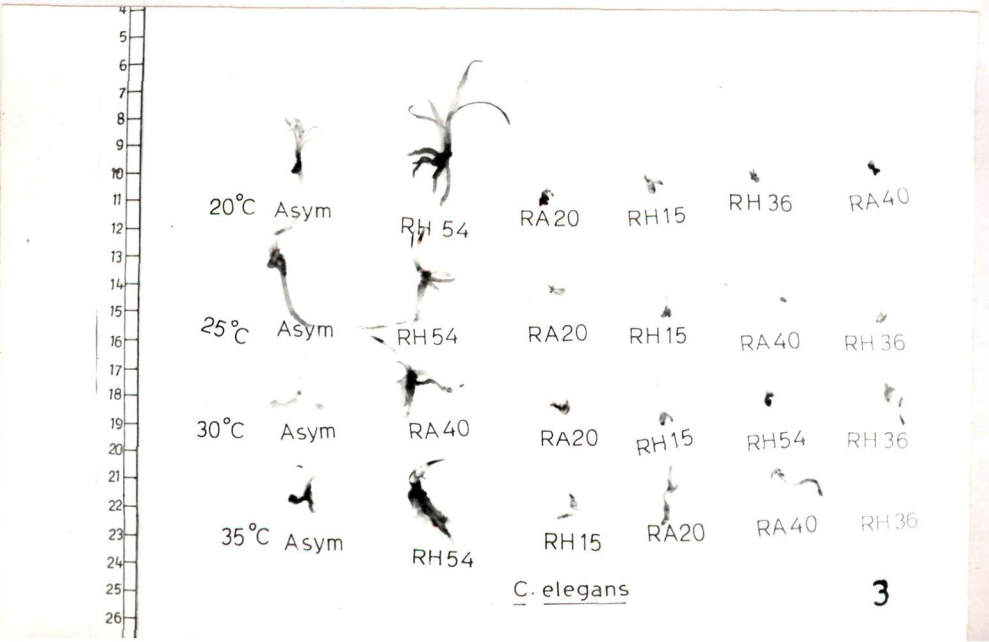
C. giganteum

1



T. alba

2



C. elegans

3

at 35°C in all the orchids using all the fungal isolates (Fig. 16-18). Significant variations were obtained between different temperatures at 1% level in relation to the growth index but the variations were insignificant between fungal isolates.

The seedlings of C. elegans incubated at 25°C temperature exhibited the highest average area of seedlings/plantlets with all the endophytes while at 35°C the area was the lowest (Table 16). The higher area of seedlings/plantlets of C. giganteum was observed at 25° and 30°C with RH15, RA20, RA40 and RH36, RH54 isolates, respectively. Similarly, area of seedlings/plantlets was also more in T. alba at 25° and 30°C temperatures by RH15, RH54, RA20, RA40 and RH36, respectively (Table 16). Statistically significant variations were observed at 5% level between different fungal isolates in relation to the seedling growth.

The formation and development of leaves and roots also varied with different temperature treatments using different mycorrhizal fungi (Tables 17 and 18). The highest average number and area of leaf primordia/leaves was noticed at 25°C temperature in C. elegans, C. giganteum and T. alba with various fungal isolates (Table 17).

Though the average number of rhizoids/roots in C. elegans was maximum at 20°C temperature in all the five

endophytes studied, the area was higher at 25°C treatment (Table 18). The rhizoids/roots production in C. giganteum was higher at 20°C using RA20, RH54 and 25°C with RH15, RH36 and RA40 (Table 18). Thunia alba exhibited the maximum number and area of rhizoids/roots at 25°C using RH15, RA20 and RA40 and at 30°C with RH36 and RH54. The rhizoids/roots development was poor in all the species at 35°C with various endophytes (Table 18).

2) Effect of pH of the medium

The symbiotic seed germination and subsequent seedling growth of orchids were variously affected by pH levels (Plate 11, Figs. 19-21). In C. elegans, the higher seed germination was obtained at pH 3.0 using RH36, RA40 and at pH 4.0 using RH15, RH54 and RA20 strains (Fig. 19). Seed germination in C. giganteum was significantly higher at pH 4.0 using RH36, RH54 and at pH 5.0 using RH15, RA20 and RA40 isolates (Fig. 20). Thunia alba exhibited the maximum seed germination at pH 6.0 with all the fungal endophytes. However, it was lowest at pH 10.0 (Fig. 21). Seed germination was significantly different at 1% level between different pH values and at 5% level between different fungal isolates in all the species.

Seedling growth also varied with different pH levels. The higher growth index in C. elegans was recorded

Fig. 19 : Symbiotic seed germination (%) and seedling growth index of Cymbidium elegans, at different pH levels of the medium.

Fig. 19

CYMBIDIUM ELEGANS

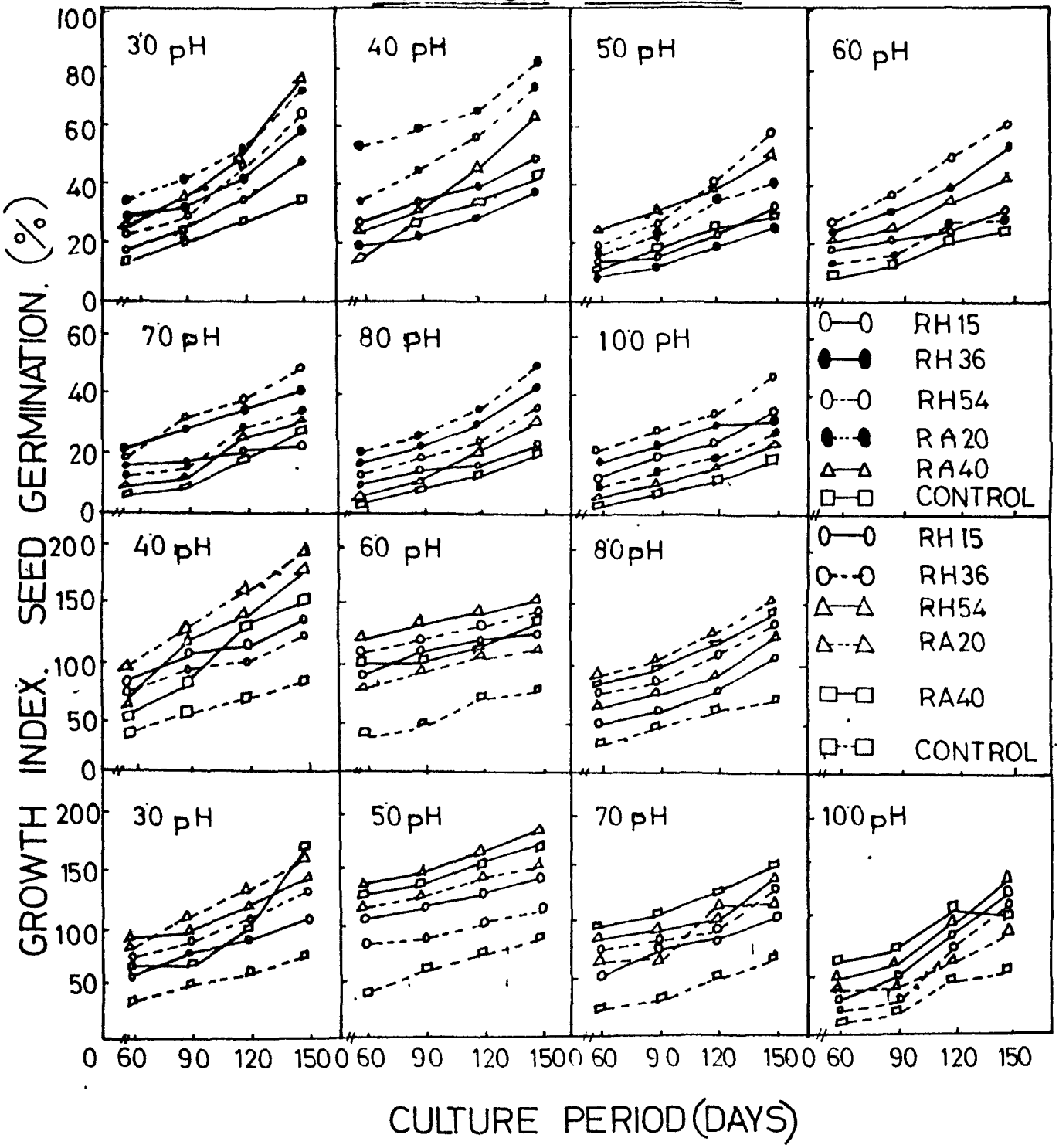


Fig. 20 : Symbiotic seed germination (%) and seedling growth index of Cymbidium giganteum, at different pH levels of the medium.

Fig. 20

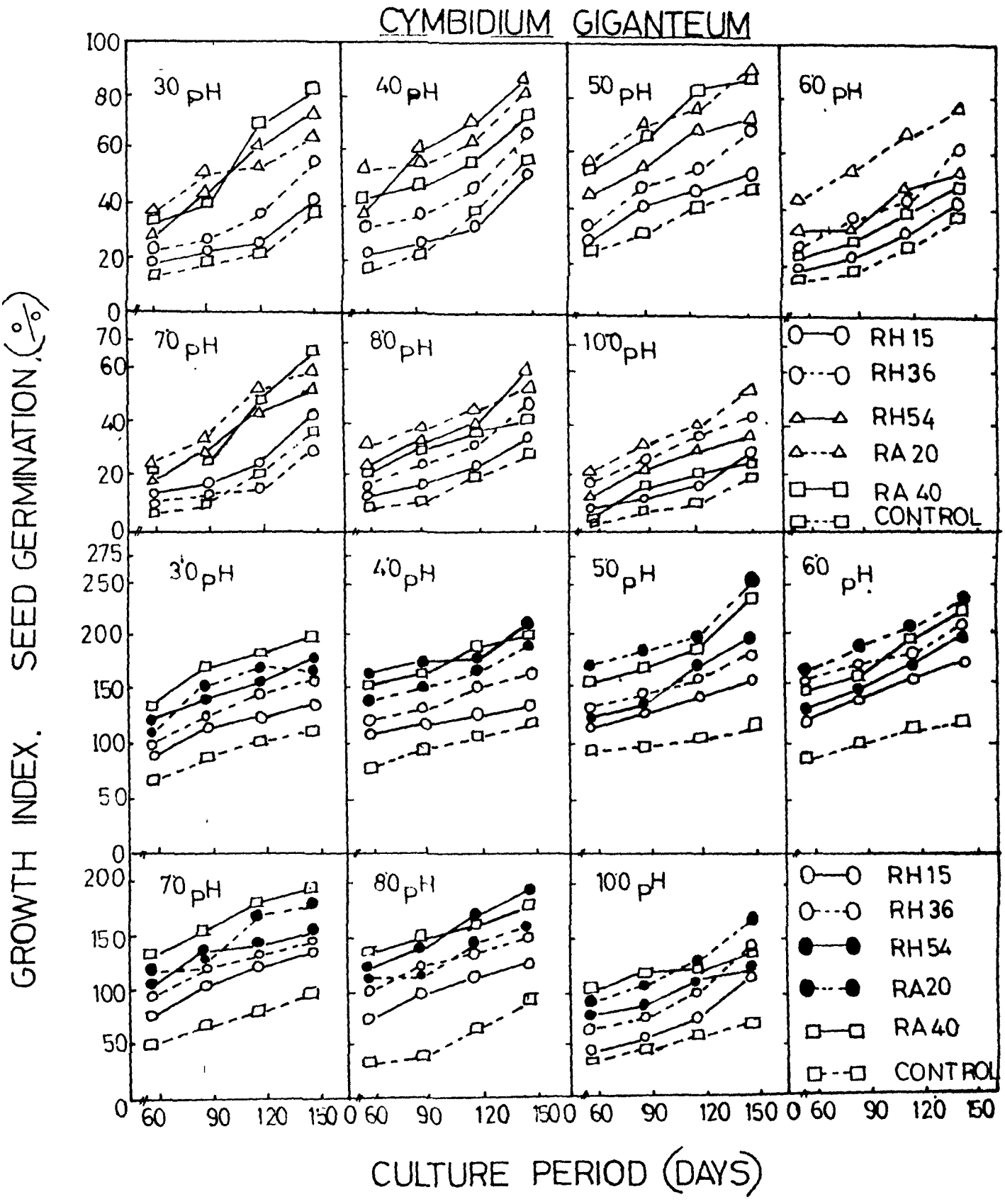

CYMBIDIUM GIGANTEUM
30 pH
40 pH
50 pH
60 pH
70 pH
80 pH
100 pH
RH 15
RH36
RH54
RA 20
RA 40
CONTROL
30 pH
40 pH
50 pH
60 pH
70 pH
80 pH
100 pH
RH 15
RH 36
RH 54
RA20
RA 40
CONTROL
GROWTH INDEX. SEED GERMINATION (%)
CULTURE PERIOD (DAYS)

Fig. 21 : Symbiotic seed germination (%) and seedling growth index of *Thunia alba*, at different pH levels of the medium.

Fig. 21

THUNIA ALBA

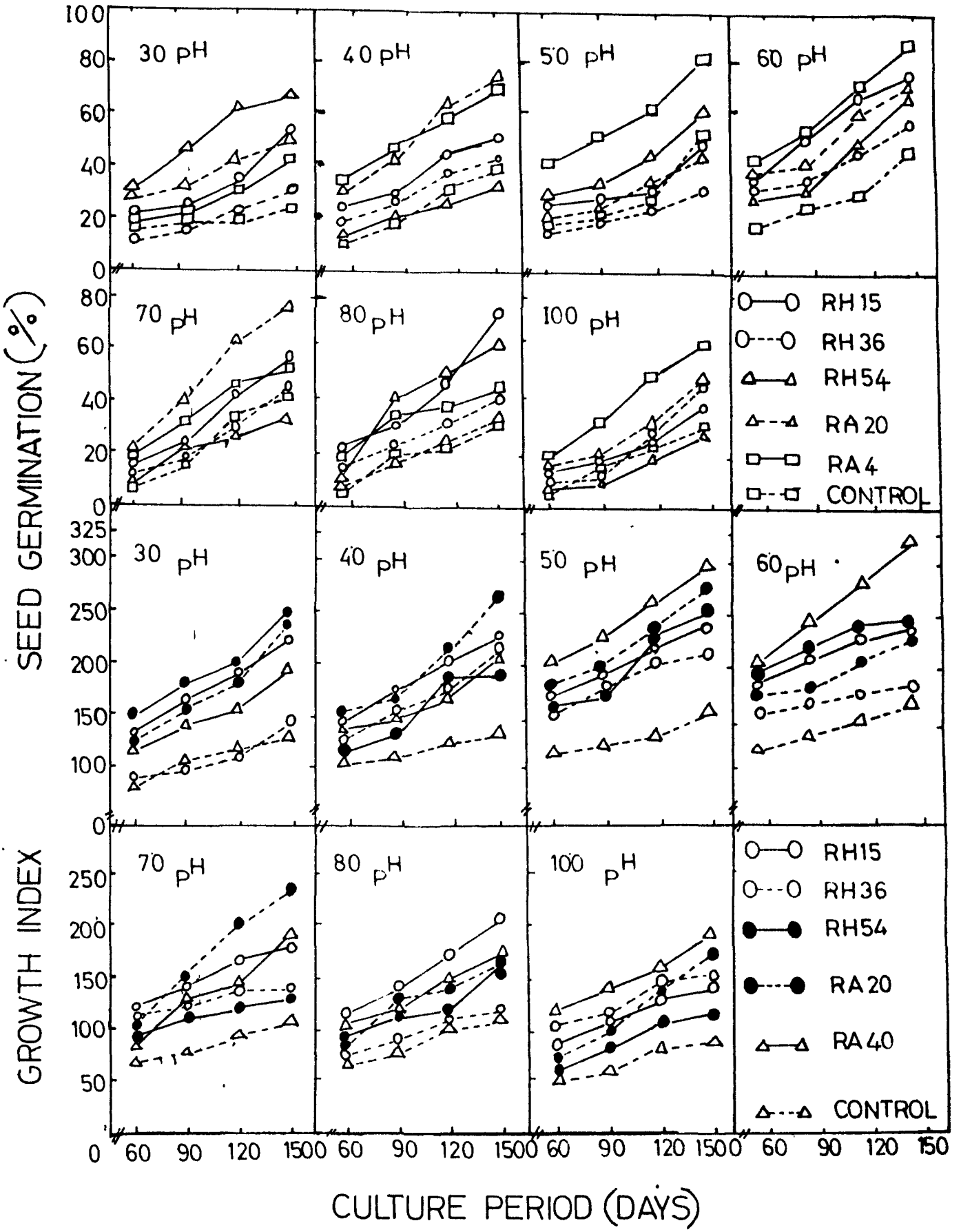


Table 19 : Effect of pH of the medium on the growth of seedlings/plantlets of orchids in symbiotic conditions after 150 days

Orchid species	Fungal isolates	Average area of seedlings/plantlets (mm^2)						
		3.0	4.0	5.0	6.0	7.0	8.0	10.0
C. elegans	RH 15	2.21	5.99	6.64	3.12	3.21	1.78	3.57
	RH 36	3.07	4.41	5.86	6.54	4.14	3.93	3.18
	RH 54	3.94	9.68	12.95	9.01	4.58	3.22	4.98
	RA 20	10.30	10.75	7.69	3.46	3.40	4.86	2.16
	RA 40	9.38	6.71	9.71	3.83	3.00	2.89	3.02
	Control	1.98	4.77	5.59	3.04	2.86	1.59	1.50
C. giganteum	RH 15	3.95	5.23	8.79	6.71	3.34	2.15	2.06
	RH 36	9.46	8.95	10.26	9.15	2.25	3.35	3.16
	RH 54	11.34	16.63	13.26	8.75	5.15	6.65	3.46
	RA 20	9.15	11.32	20.11	16.23	6.92	4.45	5.75
	RA 40	14.45	10.15	16.75	10.15	9.45	7.16	1.16
	Control	4.19	6.23	5.15	4.11	3.15	2.45	1.40
T. alba	RH 15	10.00	13.65	12.26	18.14	11.65	10.26	2.16
	RH 36	6.16	10.00	8.65	9.45	7.41	3.21	4.00
	RH 54	14.00	8.69	15.34	12.65	6.00	8.65	3.16
	RA 20	12.10	20.65	10.62	16.66	16.25	4.75	5.29
	RA 40	6.89	17.61	21.65	21.14	10.25	5.65	7.65
	Control	5.45	11.14	13.60	14.34	9.25	7.94	5.61

Table 20 : Effect of pH of the medium on the production of leaf primordia/leaves of orchids in symbiotic conditions after 150 days

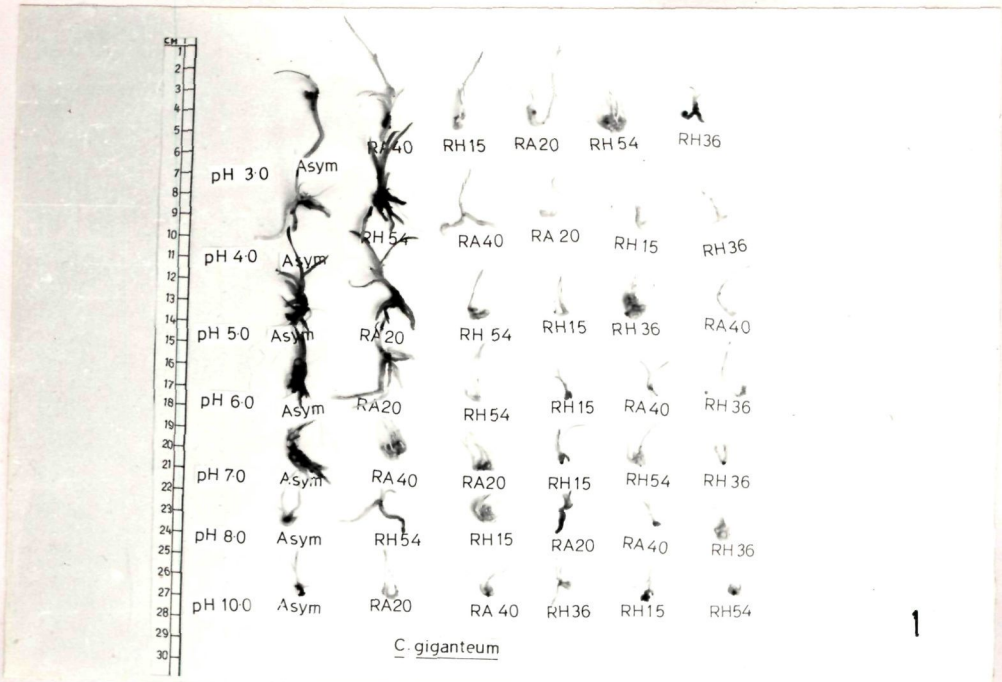
Orchid species	Fungal species	Average number and area of leaf primordia/leaves (mm^2)													
		3.0		4.0		5.0		6.0		7.0		8.0		10.0	
		No.	area	No.	area	No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	1.54	0.53	1.85	0.96	1.36	0.95	1.29	0.54	1.16	0.35	0.60	0.26	0.67	0.27
	RH 36	1.65	0.64	2.11	0.80	1.45	0.79	1.44	0.42	1.28	0.41	0.89	0.34	0.80	0.64
	RH 54	2.06	0.78	1.75	0.75	1.55	0.66	1.59	0.61	1.36	0.30	0.80	0.50	0.86	0.44
	RA 20	2.15	0.89	2.34	1.17	1.67	0.70	1.67	0.75	1.69	0.56	0.96	0.38	1.17	0.53
	RA 40	1.77	0.96	2.24	0.90	1.37	0.89	1.80	0.79	1.33	0.82	1.15	0.44	1.25	0.69
	Control	1.50	0.48	2.17	0.57	1.23	0.65	1.12	0.39	1.28	0.28	0.95	0.25	0.90	0.26
C. giganteum	RH 15	1.23	1.27	1.95	1.33	2.15	2.17	1.76	1.31	0.90	1.16	0.51	0.90	0.82	0.71
	RH 36	1.45	1.67	2.16	1.86	1.97	2.72	1.54	1.51	1.15	1.27	0.67	1.12	1.11	0.45
	RH 54	1.63	1.49	2.28	1.25	2.90	2.98	1.82	1.62	1.23	1.41	0.98	1.45	1.16	0.56
	RA 20	1.78	1.82	1.89	1.30	1.98	3.08	1.96	1.66	0.99	1.71	1.17	1.67	1.21	0.67
	RA 40	1.88	1.96	1.96	1.46	2.11	2.19	1.90	1.84	1.35	1.33	1.26	1.19	1.96	0.71
	Control	1.32	1.37	1.57	1.34	1.78	1.95	1.38	1.44	0.80	1.30	0.91	0.88	0.80	0.59
T. alba	RH 15	1.16	0.64	1.89	1.11	2.19	1.72	2.29	2.51	0.57	0.45	0.61	0.35	1.26	0.16
	RH 36	1.25	0.57	1.36	1.21	2.39	1.25	2.61	2.79	0.67	0.62	0.72	0.64	1.45	0.19
	RH 54	1.67	0.68	1.48	1.36	2.11	1.34	2.54	2.65	1.17	0.75	0.41	0.65	1.36	0.21
	RA 20	1.49	0.91	1.55	1.45	2.32	1.55	2.64	2.91	1.96	0.84	1.12	0.43	1.55	0.26
	RA 40	1.55	0.80	1.71	1.58	1.85	1.63	2.78	3.15	0.49	0.91	1.14	0.53	0.61	0.25
	Control	1.19	0.57	1.57	1.29	1.63	1.68	2.46	2.15	0.86	0.71	0.54	0.43	0.48	0.19

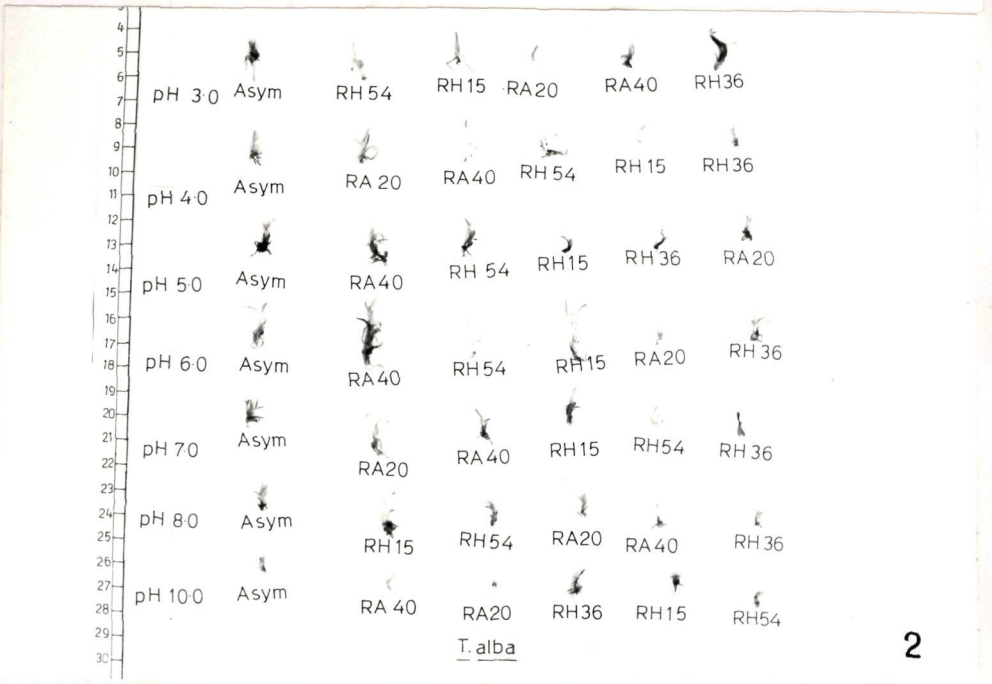
Table 21 : Effect of pH of the medium on the production of rhizoids/roots of orchids in symbiotic conditions after 150 days

Orchid species	Fungal species	Average number and area of rhizoids/roots (mm^2)											
		3.0		4.0		5.0		6.0		7.0		8.0		10.0	
		No.	area	No.	area	No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	10.15	0.12	10.00	0.21	12.45	0.35	9.62	0.06	8.23	0.09	5.17	0.08	5.25	0.009
	RH 36	8.54	0.17	12.25	0.26	13.64	0.41	7.54	0.16	7.65	0.08	6.65	0.06	5.17	0.01
	RH 54	8.95	0.21	13.65	0.22	14.00	0.45	8.63	0.23	8.89	0.06	7.92	0.07	6.22	0.04
	RA 20	9.66	0.25	10.60	0.19	13.45	0.40	9.25	0.24	10.00	0.05	7.68	0.12	6.00	0.02
	RA 40	10.25	0.20	11.66	0.30	14.55	0.39	10.69	0.20	11.00	0.12	8.45	0.15	7.00	0.05
	Control	7.67	0.15	8.65	0.25	10.82	0.29	9.64	0.12	8.65	0.04	5.15	0.09	6.00	0.03
C. giganteum	RH 15	12.00	0.26	10.65	0.54	11.45	0.82	7.95	0.65	10.00	0.89	7.75	0.32	6.32	0.14
	RH 36	14.00	0.35	9.28	0.63	9.40	0.79	7.62	0.49	8.49	1.05	8.89	0.45	7.46	0.25
	RH 54	13.96	0.40	11.65	1.15	7.76	1.22	8.11	0.86	7.32	0.54	7.76	0.55	5.49	0.36
	RA 20	12.45	0.25	10.29	1.36	8.00	1.56	8.25	1.13	9.64	1.23	8.25	0.61	8.85	0.41
	RA 40	15.00	0.36	8.65	1.23	10.64	1.30	7.00	1.25	7.45	1.00	8.00	0.49	6.99	0.35
	Control	11.15	0.20	8.85	0.50	9.96	0.78	6.66	0.25	7.35	0.24	5.45	0.32	4.44	0.34
T. alba	RH 15	1.65	0.23	1.82	0.25	1.76	0.53	2.25	1.05	1.61	0.35	1.32	0.23	0.78	0.12
	RH 36	1.48	0.26	2.00	0.36	1.50	0.74	2.40	1.03	1.78	0.46	1.45	0.40	0.95	0.09
	RH 54	1.60	0.37	1.75	0.41	2.35	0.45	3.17	0.94	1.22	0.49	1.63	0.56	1.05	0.15
	RA 20	1.77	0.48	1.86	0.54	2.25	0.53	2.29	1.15	1.45	0.55	1.22	0.50	0.82	0.17
	RA 40	2.07	0.59	2.16	0.50	2.11	0.64	2.30	0.82	1.67	0.38	1.54	0.62	1.22	0.23
	Control	1.44	0.46	1.54	0.18	2.14	0.32	2.17	1.14	1.28	0.32	1.27	0.28	0.95	0.14

Plate 11 : Symbiotic seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), at different pH levels of the medium.

PLATE 11


pH 3·0 Asym RA40 RH15 RA20 RH54 RH36
pH 4·0 Asym RH54 RA40 RA20 RH15 RH36
pH 5·0 Asym RA20 RH54 RH15 RH36 RA40
pH 6·0 Asym RA20 RH54 RH15 RA40 RH36
pH 7·0 Asym RA40 RA20 RH15 RH54 RH36
pH 8·0 Asym RH54 RH15 RA20 RA40 RH36
pH 10·0 Asym RA20 RA40 RH36 RH15 RH54
C. giganteum
1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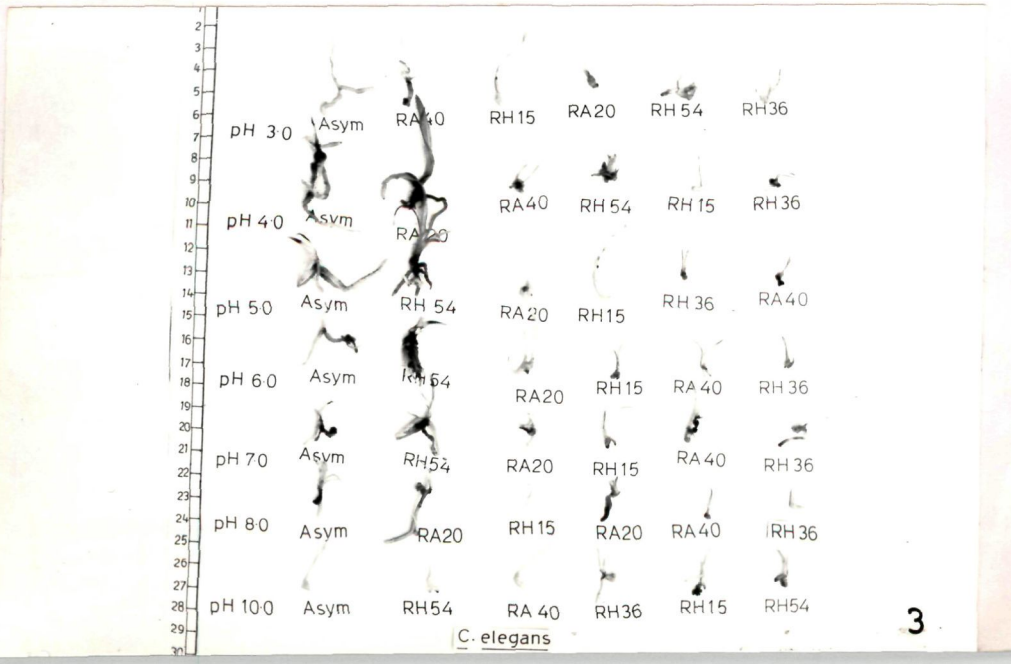
pH 4·0 Asym RA20 RA40 RH54 RH15 RH36
pH 5·0 Asym RA40 RH54 RH15 RH36 RA20
pH 6·0 Asym RA40 RH54 RH15 RA20 RH36
pH 7·0 Asym RA20 RA40 RH15 RH54 RH36
pH 8·0 Asym RH15 RH54 RA20 RA40 RH36
pH 10·0 Asym RA40 RA20 RH36 RH15 RH54
T. alba
2


pH 3·0 Asym RA40 RH15 RA20 RH54 RH36
pH 4·0 Asym RA20 RA40 RH54 RH15 RH36
pH 5·0 Asym RH54 RA20 RH15 RH36 RA40
pH 6·0 Asym RH54 RA20 RH15 RA40 RH36
pH 7·0 Asym RH54 RA20 RH15 RA40 RH36
pH 8·0 Asym RA20 RH15 RA20 RA40 RH36
pH 10·0 Asym RH54 RA40 RH36 RH15 RH54
C. elegans
3

at pH 3.0 using RA40; at pH 4.0 using RA20; at pH 5.0 using RH15, RH54 and at pH 6.0 using RH36 strains (Fig. 19). On the other hand, the growth index was higher at pH 5.0 and 6.0 with all the symbionts in case of C. giganteum (Fig. 20). T. alba showed better growth index at pH 4.0 with RH36 and RA20; at pH 5.0 with RH54 and RA40 and at pH 6.0 with RH15, than the other pH values (Fig. 21). Significant variations were observed at 1% level between various pH values and at 5% level between different fungal endophytes in relation to the growth index in all the species except C. elegans.

The average area of seedlings/plantlets was enhanced within the pH range of 4.0 - 6.0 but it decreased above pH 8.0 in all the cases, using different fungal isolates (Table 19). The area of seedlings/plantlets was statistically significant at 5% level between different pH values and different fungal isolates.

The highest production of leaf primordia/leaves occurred at pH 4.0, 5.0 and 6.0 in C. elegans, C. giganteum and T. alba, respectively with all the endophytes while, minimum was recorded at 10.0 pH (Table 20).

The average number and area of rhizoids/roots was higher at pH 5.0 and 6.0 in C. elegans and T. alba, respectively with all the mycorrhizal fungi. The number and area of rhizoids/roots were maximum at pH 3.0 and 5.0,

respectively in case of C. giganteum in all the fungal symbionts (Table 21).

3) Effect of light

a) Effect of light intensity

Seed germination and subsequent seedling growth under different light intensities varied with different mycorrhizal fungi (Plate 12, Figs. 22-24). The percentage of seed germination in C. elegans was maximum at 1500 lux light using RH36, RH54 isolates and at 5000 lux light using RH15. However, with RA20 and RA40, highest seed germination was recorded under dark conditions in C. elegans. The same fungal strains RA20 and RA40 exhibited better seed germination at 1500 lux light intensity in case of C. giganteum (Figs. 22 and 23). Thunia alba, however, showed significantly higher germination of seeds in the dark than in the light conditions with all the fungal endophytes (Fig. 24). Statistically significant variations were obtained at 1% level between different light intensities and fungal isolates in relation to the seed germination. Seedling growth also differed in different light intensities in all the species (Figs. 22-24, Table 22). The higher growth index was recorded using RH36, RH54 and RA20 at 1500 lux light and using RH15 and RA40 at 3000 lux light intensity. On the other hand, uninoculated seedlings had more growth index under 1500 lux light intensity in C. elegans (Fig.. 22). The growth index was higher at 1500 lux light than the other light

Fig. 22 : Symbiotic seed germination (%) and seedling growth index of Cymbidium elegans, at different light intensities.

Fig 22

CYMBIDIUM ELEGANS

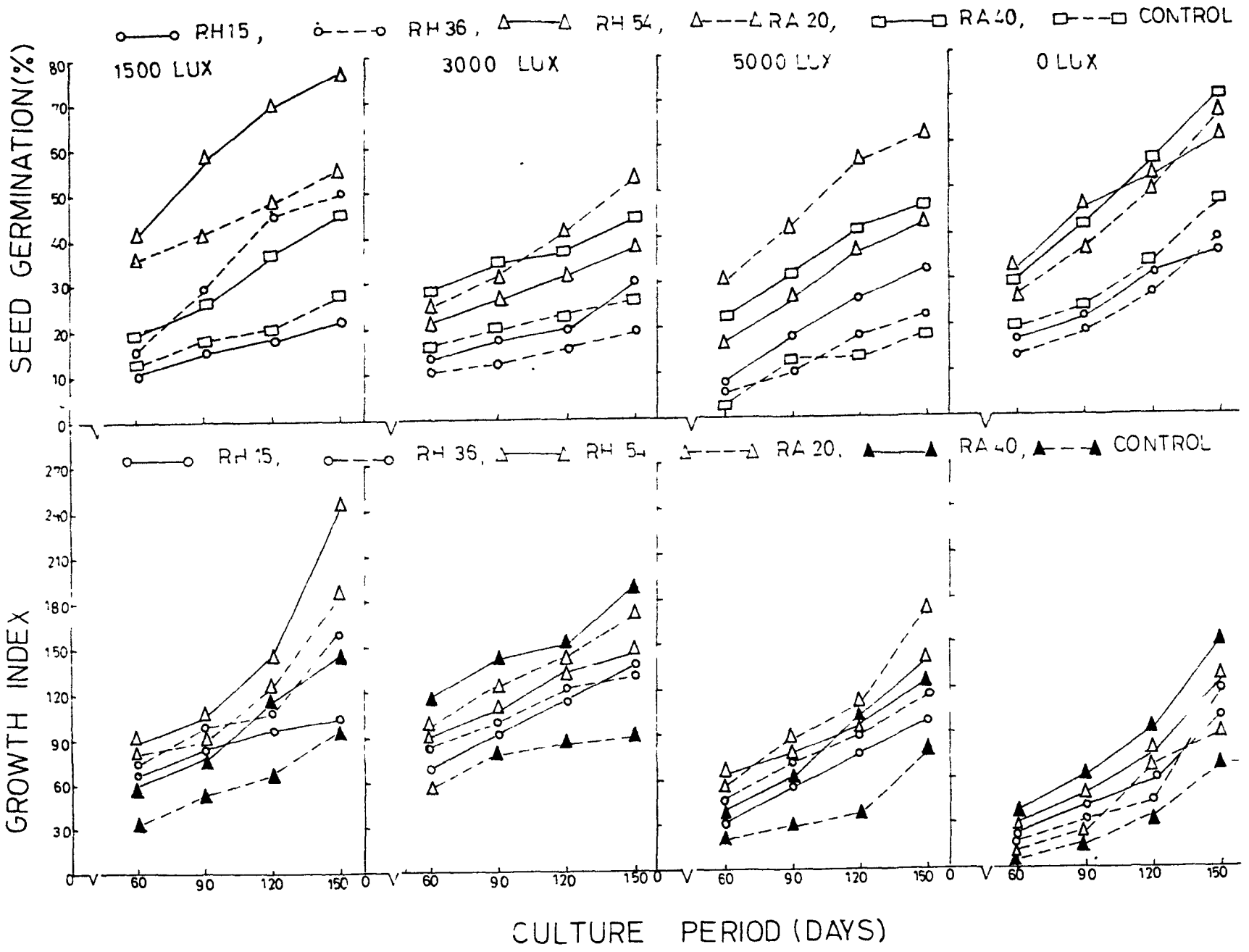


Fig. 23 : Symbiotic seed germination and seedling growth index of Cymbidium giganteum, at different light intensities.

Fig. 23

CYMBIDIUM GIGANTEUM

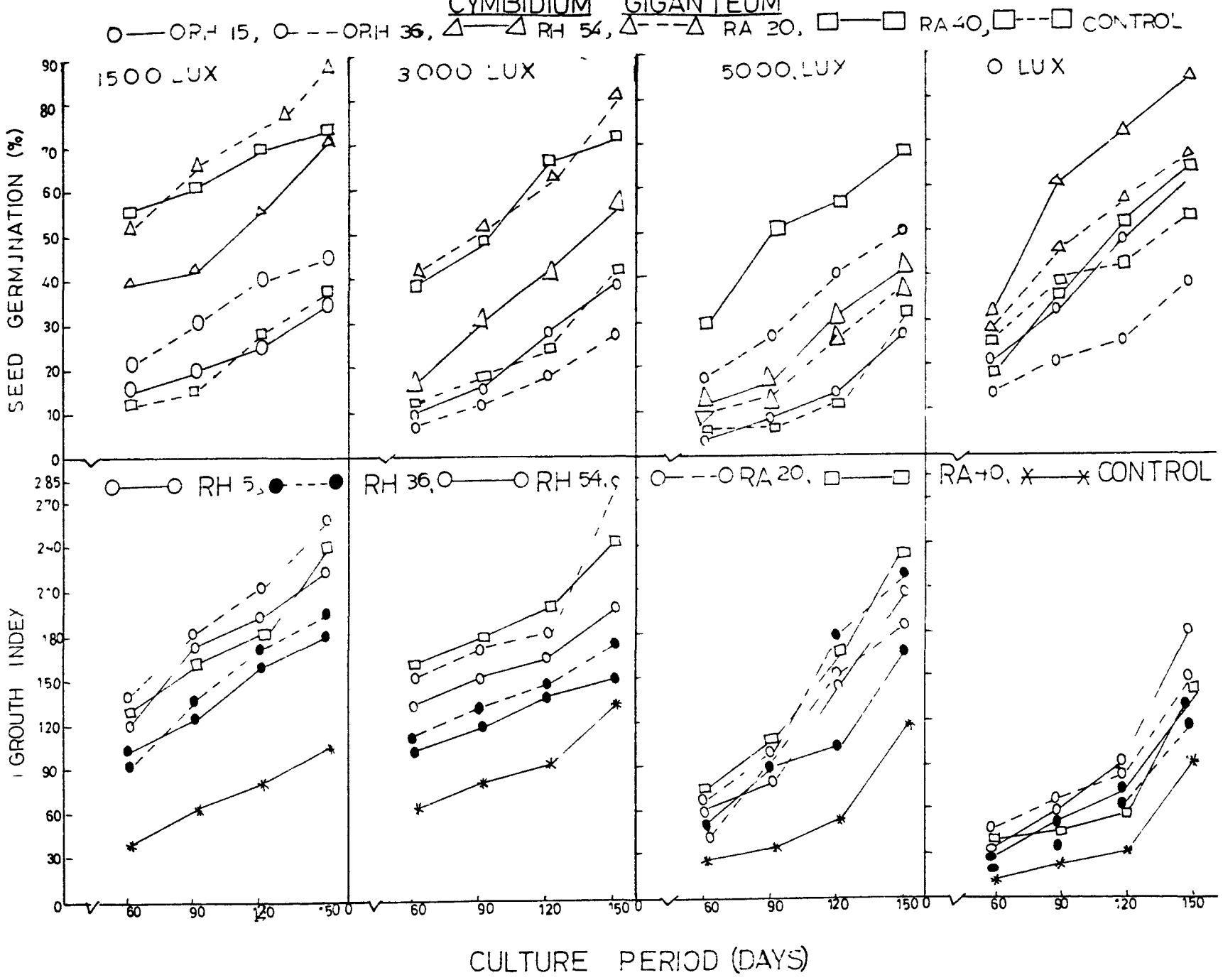


Fig. 24 : Symbiotic seed germination (%) and seedling growth index of *Thunia alba*, at different light intensities.

Fig. 24

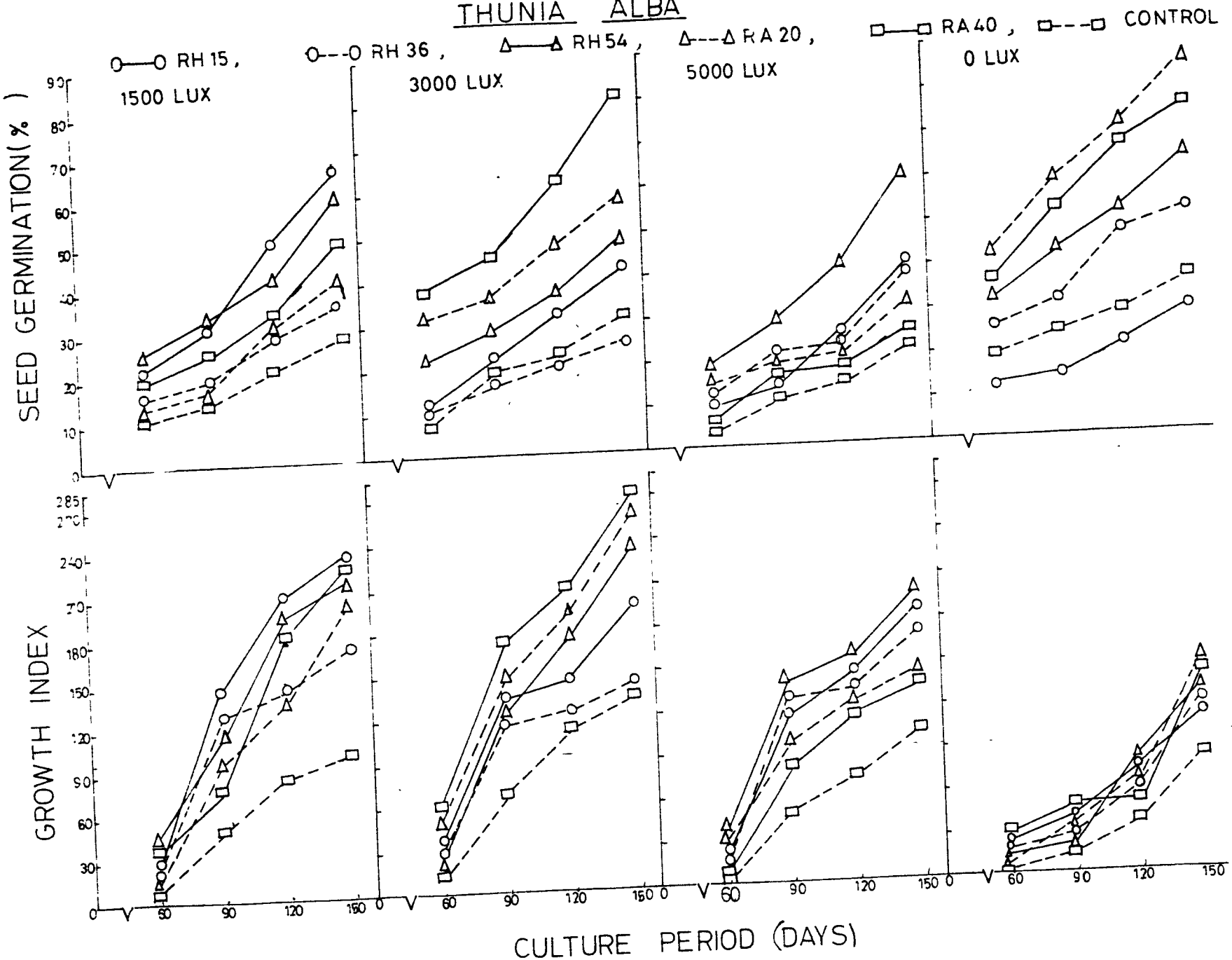

THUNIA ALBA
RH 15, 1500 LUX
RH 36, 3000 LUX
RH 54, 5000 LUX
RA 20, RA 40, CONTROL, 0 LUX
SEED GERMINATION (%)
GROWTH INDEX
CULTURE PERIOD (DAYS)

Table 22 : Effect of light intensities on the growth of seedlings/ plantlets of orchids in symbiotic conditions after 150 days

Orchid species	Fungal isolates	Average area of seedlings/plantlets(mm^2)			
		1500 lux	3000 lux	5000 lux	0 lux
	RH 15	4.55	2.95	1.35	0.96
	RH 36	3.67	2.69	1.17	0.79
C. elegans	RH 54	5.55	3.79	2.61	1.45
	RA 20	6.78	4.51	2.24	2.00
	RA 40	5.53	3.77	2.35	1.11
	Control	2.64	1.36	1.12	1.00
	RH 15	8.76	13.45	6.75	4.36
	RH 36	9.55	16.78	5.55	5.17
C. giganteum	RH 54	10.65	15.55	7.12	4.54
	RA 20	12.55	18.67	10.14	6.95
	RA 40	11.69	17.55	12.96	5.77
	Control	9.54	13.57	7.93	3.53
	RH 15	8.76	10.17	7.45	2.15
	RH 36	6.77	12.15	9.17	3.63
T. alba	RH 54	5.95	11.16	10.26	4.75
	RA 20	9.76	9.89	12.14	7.92
	RA 40	10.54	13.15	11.76	3.11
	Control	5.99	7.66	6.16	2.15

Table 23 : Effect of light intensities on the production of leaf primordia/leaves of orchids in symbiotic conditions after 150 days

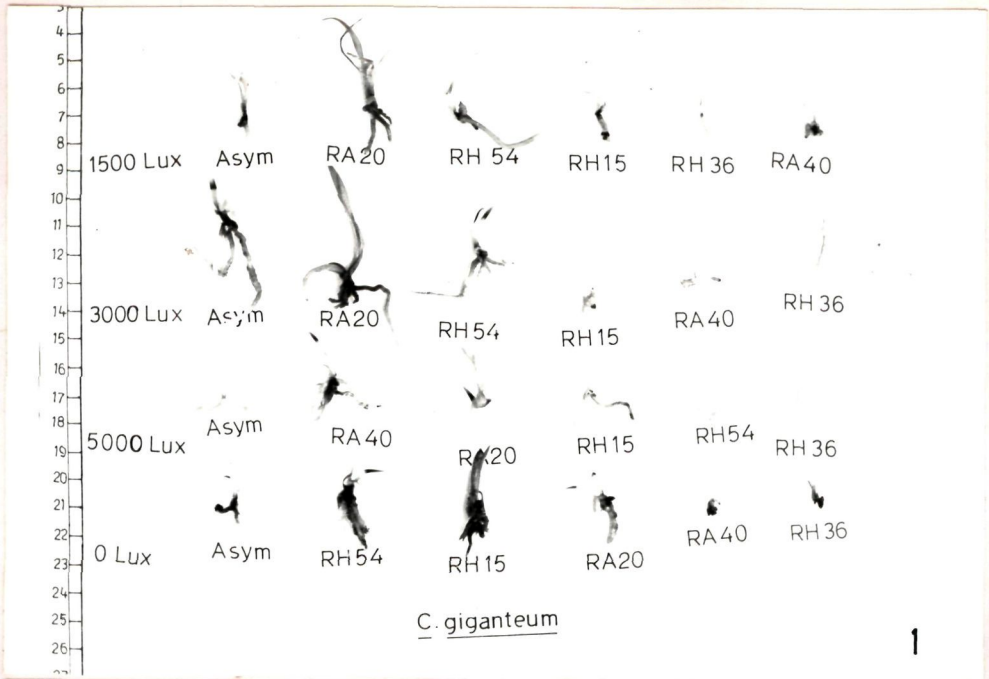
Orchid species	Fungal isolates	Average number and area of leaf primordia/leaves (mm^2)							
		1500 lux		3000 lux		5000 lux		0 lux	
		No.	area	No.	area	No.	area	No.	area
	RH 15	1.95	1.15	1.35	0.65	0.96	0.26	0.54	0.12
	RH 36	2.00	1.26	1.41	0.75	0.75	0.35	0.61	0.15
C. elegans	RH 54	2.15	1.31	1.30	0.81	0.92	0.41	0.81	0.21
	RA 20	2.25	1.44	1.44	0.99	1.12	0.51	0.96	0.23
	RA 40	2.31	2.05	1.61	0.75	1.25	0.44	0.87	0.27
	Control	1.87	1.29	1.51	0.47	0.85	0.28	0.71	0.20
	RH 15	1.15	0.95	1.76	1.55	1.21	1.24	0.46	0.23
	RH 36	1.21	0.87	1.85	1.63	1.25	1.35	0.51	0.46
C. giganteum	RH 54	1.36	0.96	1.75	1.77	1.00	1.41	0.66	0.53
	RA 20	1.41	1.25	2.66	1.85	1.45	1.69	0.77	0.61
	RA 40	1.27	1.31	2.54	1.90	1.67	1.35	0.83	0.78
	Control	1.16	0.96	1.49	1.61	1.11	1.19	0.57	0.35
	RH 15	1.15	0.89	2.05	2.65	1.35	1.22	0.65	0.23
	RH 36	1.21	0.95	2.16	1.98	1.38	1.35	1.12	0.44
T. alba	RH 54	1.67	1.05	1.95	2.12	1.75	1.41	1.25	0.51
	RA 20	1.54	0.79	2.32	2.96	1.83	1.61	0.96	0.65
	RA 40	1.84	1.15	2.45	2.75	1.96	1.70	1.31	0.72
	Control	1.13	0.95	1.65	1.85	1.24	1.55	1.00	0.24

Table 24 : Effect of light intensities on the production of rhizoids/roots of orchids in symbiotic conditions after 150 days

Orchid species	Fungal isolates	Average number and area of rhizoids/roots (mm^2)							
		1500 lux		3000 lux		5000 lux		0 lux	
		No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	12.11	0.26	11.75	0.29	6.76	0.12	8.95	0.01
	RH 36	14.65	0.29	9.56	0.26	8.95	0.15	7.65	0.05
	RH 54	14.00	0.35	10.25	0.37	7.44	0.13	9.99	0.06
	RA 20	17.66	0.38	12.45	0.35	9.54	0.19	10.44	0.17
	RA 40	19.92	0.41	11.96	0.38	10.12	0.16	12.26	0.11
	Control	10.65	0.28	8.15	0.21	4.54	0.14	7.65	0.02
C. giganteum	RH 15	11.12	0.87	13.55	0.72	9.11	0.31	12.75	0.08
	RH 36	14.72	0.96	14.96	0.96	8.25	0.42	10.96	0.11
	RH 54	16.92	0.69	15.32	0.76	9.67	0.51	11.77	0.15
	RA 20	18.65	1.05	16.17	0.84	10.11	0.65	13.76	0.16
	RA 40	13.11	1.11	11.14	0.95	11.45	0.55	14.95	0.09
	Control	9.76	0.45	8.59	0.33	5.75	0.28	10.54	0.07
T. alba	RH 15	1.76	0.36	2.15	0.61	1.55	0.23	1.56	0.09
	RH 36	1.26	0.45	1.96	0.77	1.29	0.41	1.45	0.07
	RH 54	2.00	0.55	2.14	0.60	1.54	0.36	1.96	0.11
	RA 20	2.15	0.69	2.42	0.85	1.69	0.55	1.55	0.15
	RA 40	2.35	0.48	2.58	0.88	1.88	0.49	1.76	0.17
	Control	1.38	0.38	1.98	0.67	1.14	0.28	1.54	0.12

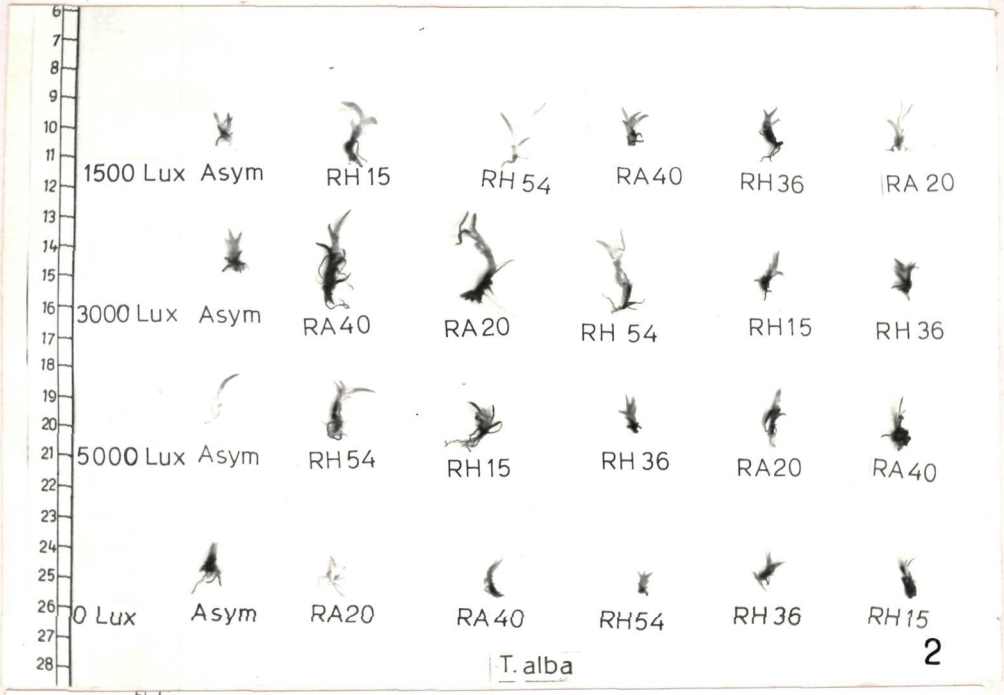
Plate 12 : Symbiotic seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), at different light intensities.

PLATE 12



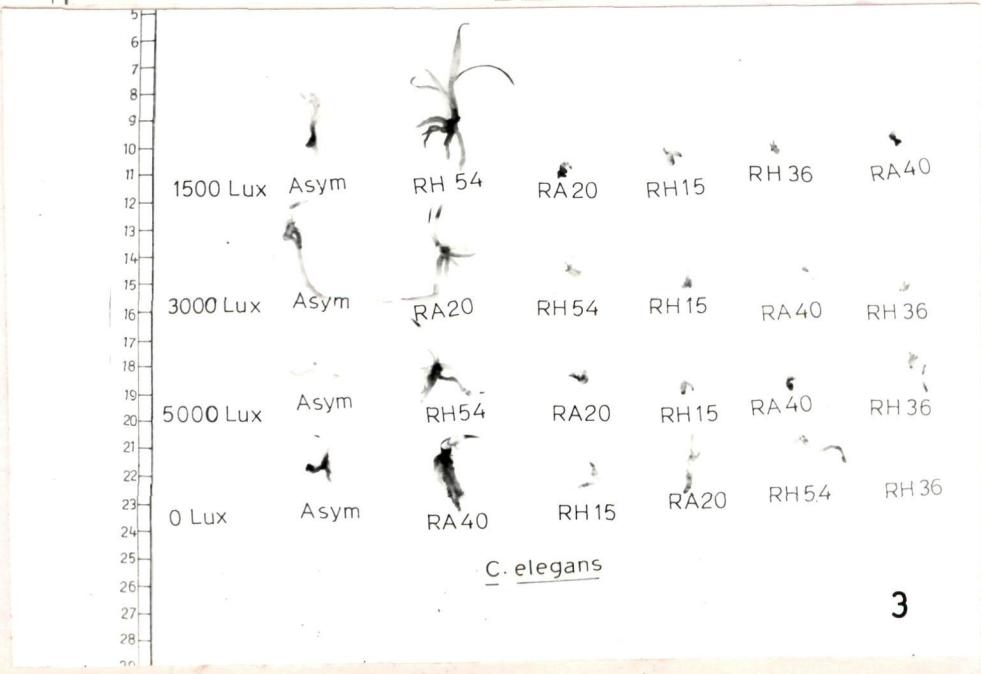
C. giganteum

1



T. alba

2



C. elegans

3

intensities in case of C. giganteum using all the fungal symbionts (Fig. 23). T. alba exhibited the higher growth index using RH15 and RH36 under 1500 lux light and using RH54, RA20 and RA40 under 3000 lux light intensity. It was the lowest in the dark conditions (Fig. 24). Significantly different growth index was found at 1% level between different light intensities though, the difference between fungal isolates was not significant in all the cases. The average area of seedlings/plantlets was significantly more under 1500 lux and 3000 lux light intensities in C. elegans and C. giganteum, respectively with all the mycorrhizal fungi. T. alba showed comparatively higher area of seedlings/plantlets at 3000 lux and 5000 lux light than the other light intensities (Table 22). The significant variations were obtained at 1% and 5% levels between different light intensities and mycorrhizal fungi respectively in relation to the area of seedlings/plantlets.

The formation and development of leaves and roots also differed at various light intensities. The maximum average number and area of leaf primordia/leaves were noticed under 1500 lux and 3000 lux light intensities in C. elegans and in C. giganteum, T. alba, respectively by all the fungal endophytes. While, minimum number and area of leaf primordia/leaves was noticed in the dark (Table 23).

Similarly, average number and area of rhizoids/roots were also highest in 1500 lux and 3000 lux light intensities in case of C. elegans and T. alba, respectively for all the five fungal symbionts. However, C. giganteum exhibited maximum production of rhizoids/roots using RH54, RA20 and RA40 under 1500 lux and using RH36 and RH15 in 3000 lux light intensities (Table 24).

b) Effect of light quality

The effect of light quality on seed germination and subsequent seedling growth of orchids with different fungal symbionts varied (Plate 13, Figs. 25-27). The higher seed germination in C. elegans was noted using RH36 and RH54 strains under red light; RA20 in green light and RH15 and RA40 endophytes in the dark conditions (Fig. 25). In case of C. giganteum, using RH36 and RA20 showed significantly better seed germination under red light than the other light of other colours.

The seed germination in C. giganteum was enhanced using RA40 in green light and using RH15, RH54 in white light (Fig. 26). Whereas, T. alba exhibited comparatively better seed germination in the blue and red light conditions with all the mycorrhizal fungi than the light of other colours (Fig. 27). Significantly variations were found at 5% level in case of C. elegans and at 1% level in C. giganteum and T. alba in

Fig. 25 : Symbiotic seed germination (%) and seedling growth index of Cymbidium elegans, under light of different qualities.

Fig 25

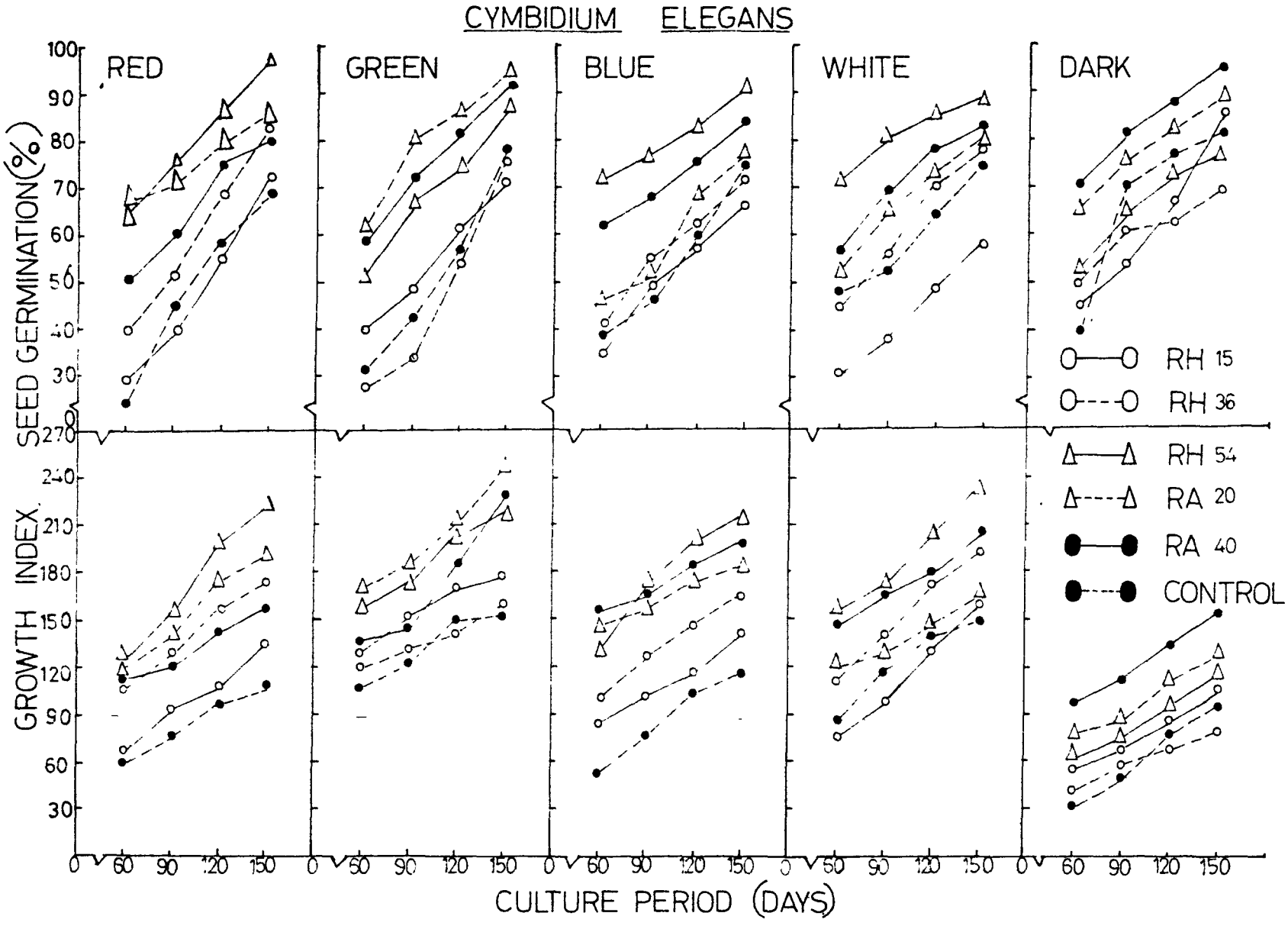

CYMBIDIUM ELEGANS
RED
GREEN
BLUE
WHITE
DARK
SEED GERMINATION(%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
RH 15
RH 36
RH 54
RA 20
RA 40
CONTROL

Fig. 26 : Symbiotic seed germination (%) and seedling growth index of Cymbidium giganteum, under light of different qualities.

Fig: 26

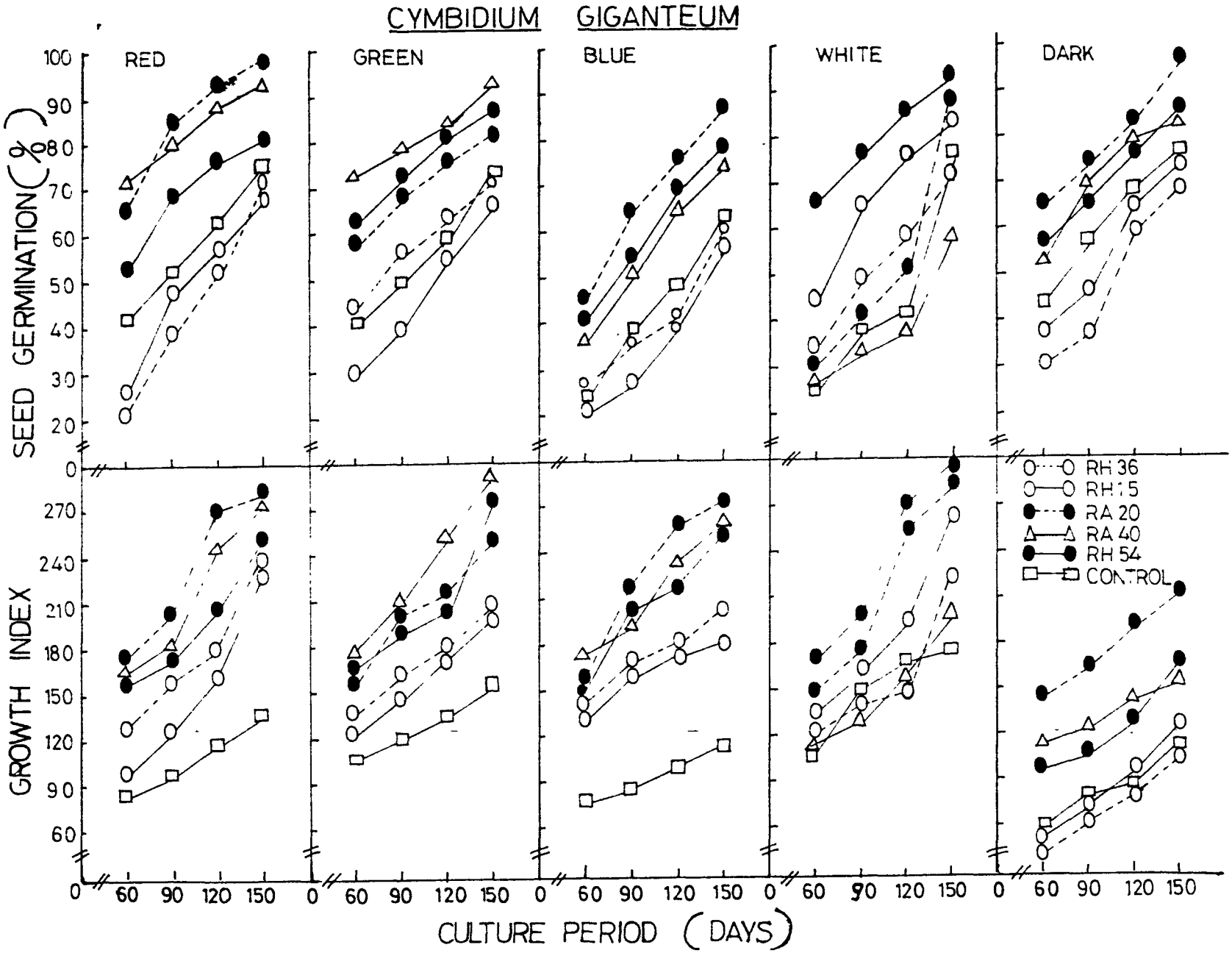

CYMBIDIUM GIGANTEUM
RED
GREEN
BLUE
WHITE
DARK
SEED GERMINATION (%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
RH 36
RH 75
RA 20
RA 40
RH 54
CONTROL

Fig. 27 : Symbiotic seed germination (%) and seedling growth index of Thunia alba, under light of different qualities.

Fig 27

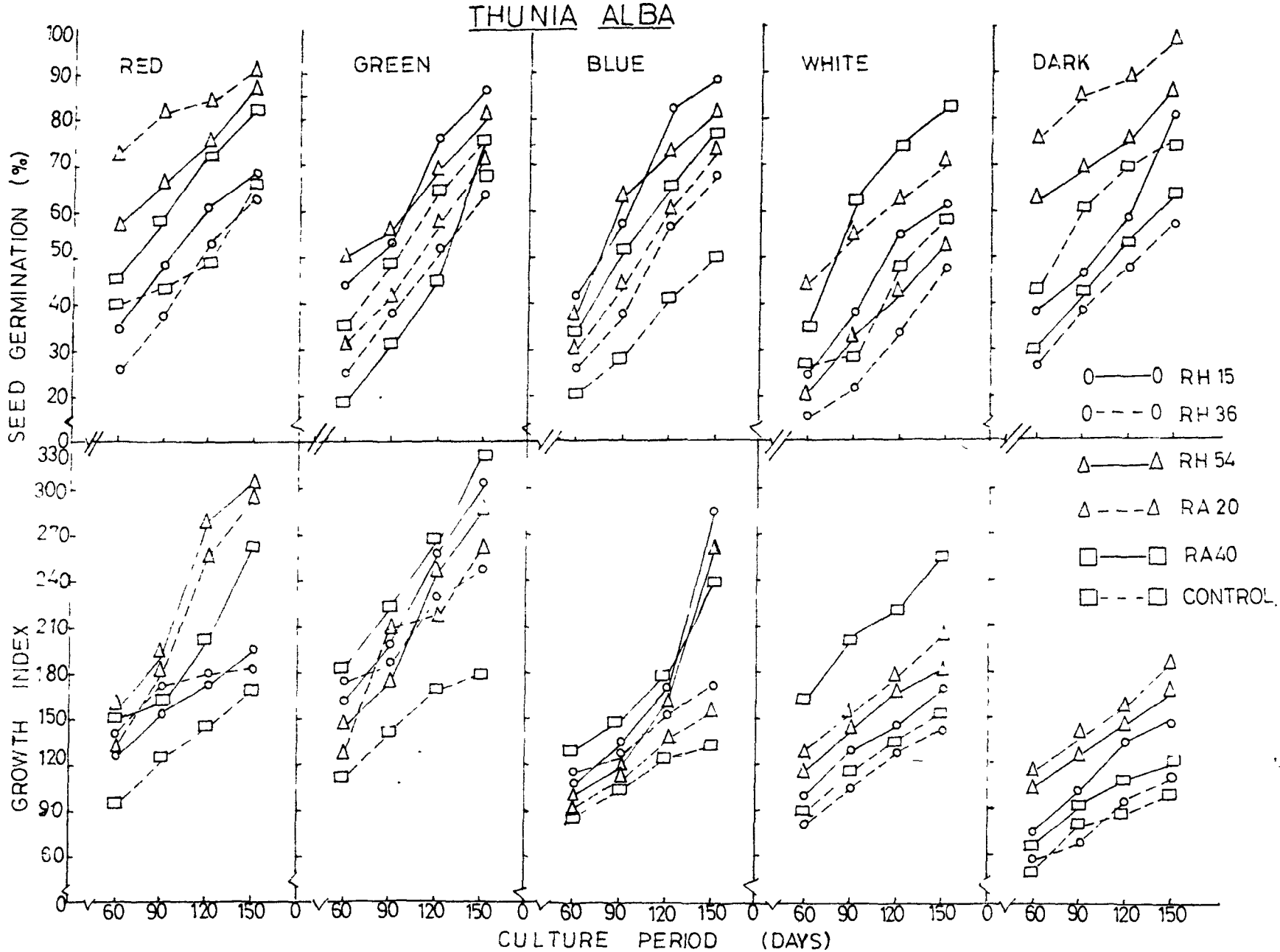

THUNIA ALBA
RED
GREEN
BLUE
WHITE
DARK
SEED GERMINATION (%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
0—0 RH 15
0- - -0 RH 36
Δ—Δ RH 54
Δ- - -Δ RA 20
□—□ RA40
□- - -□ CONTROL

Table 25 : Effect of light qualities on the growth of seedlings/ plantlets of orchids in symbiotic conditions after 150 days

Orchid species	Fungal isolates	Average area of seedlings/plantlets (mm^2)				
		red	green	blue	white	dark
C. elegans	RH 15	2.32	3.19	1.97	2.86	1.42
	RH 36	2.84	2.65	2.00	4.45	1.29
	RH 54	5.76	4.74	3.62	6.72	1.33
	RA 20	3.92	7.18	2.65	3.29	1.76
	RA 40	2.71	5.63	3.18	4.11	2.15
	Control	3.17	6.63	3.00	4.12	1.85
C. giganteum	RH 15	4.17	7.49	4.42	15.54	1.48
	RH 36	4.92	8.88	5.95	10.15	1.59
	RH 54	5.69	12.25	7.47	18.88	1.74
	RA 20	12.26	10.00	8.67	16.66	2.26
	RA 40	9.48	15.95	6.66	8.95	2.60
	Control	8.99	10.96	7.00	14.74	2.05
T. alba	RH 15	5.29	13.14	9.53	7.62	3.29
	RH 36	4.36	6.92	4.69	4.57	2.11
	RH 54	11.63	12.12	6.55	6.69	4.22
	RA 20	9.96	8.88	4.17	9.26	5.38
	RA 40	7.65	10.62	5.79	12.29	2.68
	Control	9.45	11.17	6.74	8.98	3.19

Table 26 : Effect of light qualities on the production of leaf primordia/leaves of orchids in symbiotic conditions after 150 days

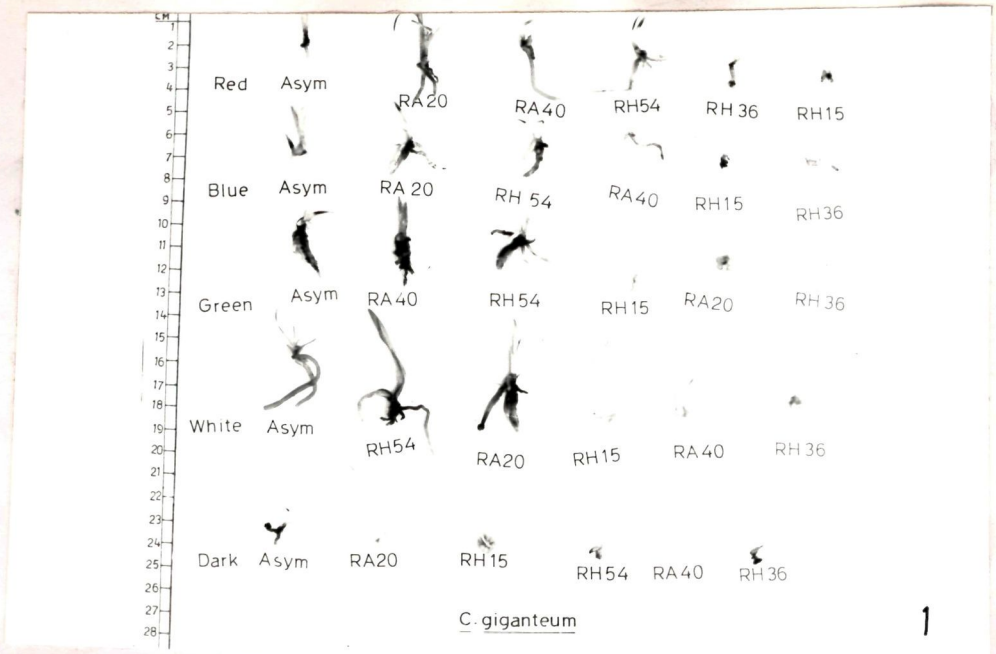
Orchid species	Fungal isolates	Average number and area of leaf primordia/leaves (mm²)									
		red		green		blue		white		dark	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	1.46	0.36	2.25	0.56	0.84	0.21	1.55	1.05	0.57	0.08
	RH 36	1.55	0.45	2.20	0.67	1.22	0.46	1.91	1.28	0.67	0.15
	RH 54	2.17	0.54	2.81	1.55	2.17	0.59	2.26	1.64	0.92	0.23
	RA 20	2.56	0.87	2.36	1.47	0.92	0.63	2.77	1.45	0.77	0.09
	RA 40	1.95	0.94	1.95	1.79	0.84	0.45	2.65	1.63	0.84	0.26
	Control	1.67	0.34	1.70	0.54	0.63	0.78	1.49	1.13	0.74	0.12
C. giganteum	RH 15	1.32	1.24	1.48	1.64	0.86	0.92	1.45	2.05	0.58	0.25
	RH 36	1.64	1.56	2.11	1.14	1.13	0.67	1.72	1.62	0.52	0.32
	RH 54	2.41	1.54	2.64	1.35	0.94	0.90	2.33	1.74	0.94	0.61
	RA 20	2.00	2.07	2.23	2.15	0.76	1.19	2.41	1.54	0.72	0.55
	RA 40	2.32	1.38	2.36	2.32	1.43	1.24	2.59	2.32	0.96	0.79
	Control	1.71	1.13	1.32	1.49	0.72	0.78	2.00	1.59	0.44	0.40
T. alba	RH 15	0.77	0.47	2.41	1.25	0.86	0.58	1.57	1.35	0.51	0.16
	RH 36	1.15	0.57	3.25	1.44	1.20	0.63	1.62	1.75	0.63	0.23
	RH 54	0.94	0.80	2.65	1.61	0.95	0.79	1.45	1.54	0.85	0.41
	RA 20	1.26	0.67	2.54	1.92	2.00	0.92	1.66	1.62	0.79	0.33
	RA 40	1.47	1.26	3.71	1.15	1.79	1.12	2.79	1.59	0.67	0.56
	Control	1.12	0.66	1.96	0.89	1.00	0.70	1.30	1.32	0.45	0.28

Table 27 : Effect of light qualities on the production of rhizoids/roots of orchids in symbiotic conditions after 150 days

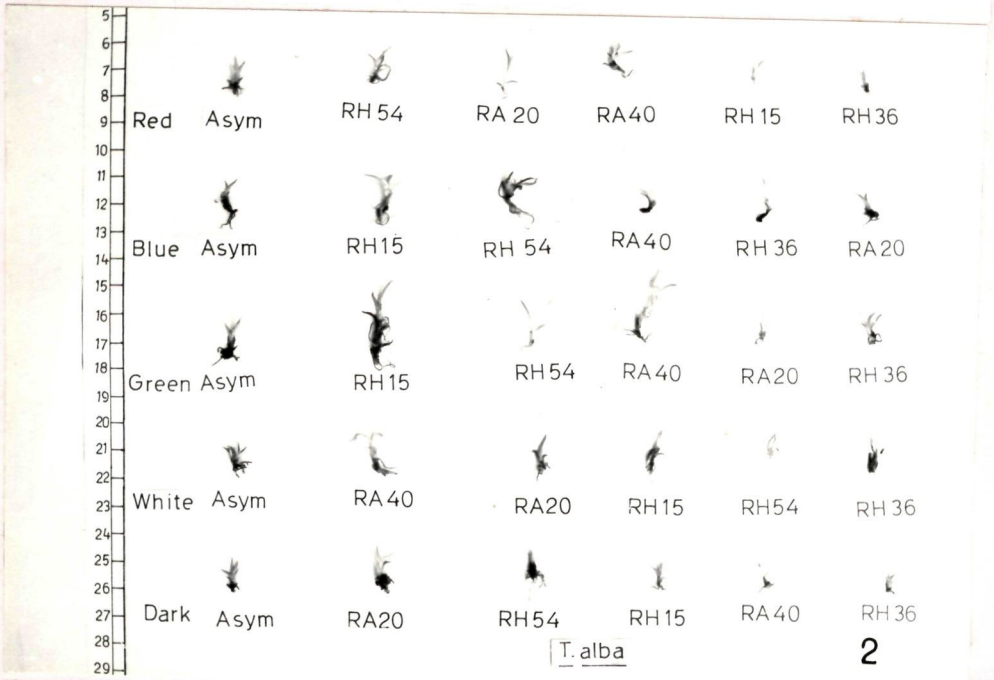
Orchid species	Fungal isolates	Average number and area of rhizoids/roots (mm^2)									
		red		green		blue		white		dark	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	7.92	0.09	9.67	0.19	5.63	0.06	8.74	0.05	7.72	0.01
	RH 36	11.25	0.07	8.88	0.22	4.92	0.12	7.45	0.12	8.92	0.04
	RH 54	8.45	0.12	9.77	0.17	8.26	0.15	9.46	0.15	9.67	0.03
	RA 20	9.67	0.21	10.16	0.24	7.75	0.07	10.15	0.14	11.25	0.05
	RA 40	9.41	0.25	12.25	0.26	9.79	0.09	12.14	0.09	13.95	0.07
	Control	7.67	0.11	9.45	0.29	5.62	0.04	5.44	0.14	7.97	0.02
C. giganteum	RH 15	13.17	0.13	10.17	0.36	7.45	0.71	10.12	1.21	9.65	0.08
	RH 36	9.45	0.19	12.25	0.44	8.92	0.54	12.12	1.15	12.20	0.09
	RH 54	8.65	0.23	13.16	0.67	9.90	0.67	11.10	0.98	10.17	0.14
	RA 20	10.17	0.35	15.17	1.12	10.35	0.92	14.16	1.25	13.65	0.13
	RA 40	12.15	0.19	12.20	1.00	11.17	0.86	14.65	0.99	14.90	0.07
	Control	8.88	0.12	9.79	0.66	9.00	0.60	8.45	0.85	10.17	0.08
T. alba	RH 15	1.78	0.19	2.17	1.14	1.45	0.56	1.50	0.66	1.47	0.06
	RH 36	1.82	0.26	2.45	0.99	0.96	0.54	1.75	1.15	1.35	0.05
	RH 54	1.96	0.45	2.66	1.63	1.29	0.79	1.67	0.77	1.78	0.04
	RA 20	2.65	0.56	2.32	1.45	1.40	0.84	1.95	1.29	1.95	0.08
	RA 40	1.88	0.61	2.17	1.13	1.63	0.96	1.78	1.64	1.63	0.06
	Control	1.47	0.35	1.85	1.00	0.65	0.75	1.22	0.54	1.44	0.05

Plate 13 : Symbiotic seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), under light of different qualities.

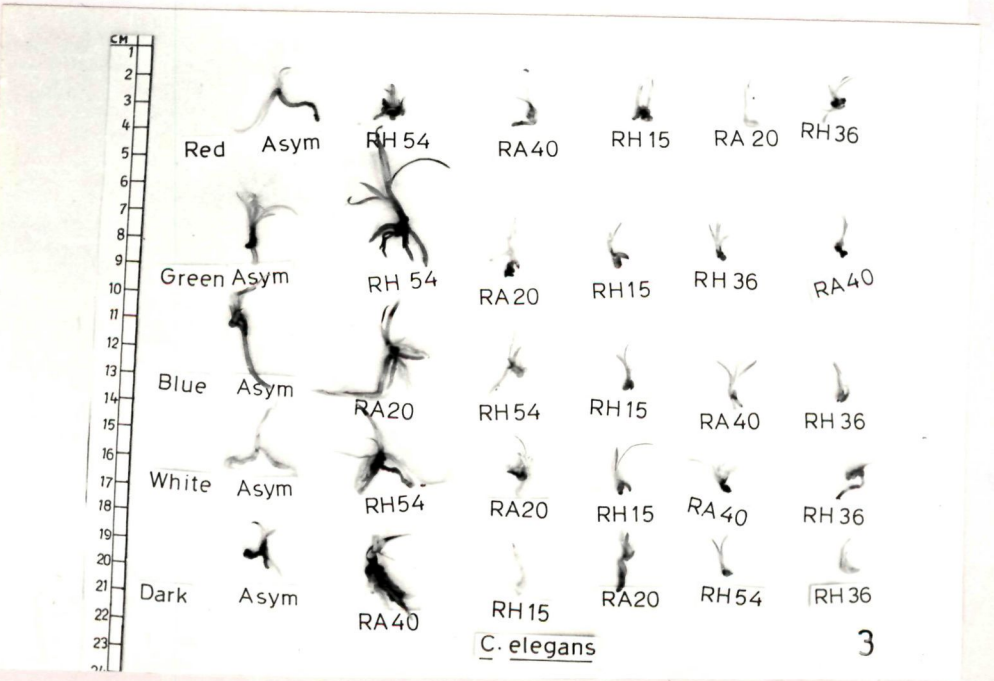
PLATE 13



C. giganteum 1



T. alba 2



C. elegans 3

relation to the seed germination.

Growth index in C. elegans was increased under green and white light with RH15, RA20, RA40 and with RH36, RH54 isolates, respectively (Fig. 25). On the other hand, mycorrhizal fungi promoted growth index in the red, green and white light conditions in case of C. giganteum (Fig. 26). The growth index was significantly higher in the red and green light than the light of other colours using all the fungal symbionts in T. alba. The controls, in this case, however, showed the maximum growth indices in the green and white light in all the species (Figs. 25-27). The growth index was significantly different at 5% level between fungal isolates and light qualities in all the cases.

The average area of seedlings/plantlets also differed with light of different colours (Table 25). The higher average area of seedlings/plantlets was recorded under the green and white light in all the species (Table 25).

The average number and area of leaf primordia/leaves recorded were maximum and minimum under the green light and dark conditions, respectively with all the fungal symbionts in all the species (Table 26). Likewise, average number and area of rhizoids/roots were also higher in the green light than the light of other colours in C. elegans and T. alba. C. giganteum exhibited the highest production of rhizoids/

roots under the white light condition (Table 27).

o) Effect of photoperiod

The results showed that the percentage of seed germination and subsequent seedling development of orchids in symbiotic conditions varied in different photoperiods (Plate 14, Figs. 28-30). The seed germination in C. elegans increased using RH15 and RA40 under 8 hr; RA20 under 12 hr; RH36 under 20 hr and RH54 under 24 hr photoperiods (Fig. 28). In case of C. giganteum, 8 and 12 hr photoperiods showed significantly better seed germination than the other photoperiods (Fig. 29). Thunia alba showed the best seed germination in 8 hr photoperiod with all the mycorrhizal fungi (Fig. 30). The germination decreased at 24 hr photoperiod in all the species (Figs. 28-30). Significant variations were obtained between different photoperiods and mycorrhizal fungi in relation to the seed germination in all the cases.

Seedling growth also differed at different photoperiods. The maximum growth index was noted under 12 hr photoperiod by all the fungal symbionts in all the species while, the minimum was recorded in 24 hr photoperiod (Figs. 28-30). The average area of seedlings/plantlets in C. elegans was higher using RH36, RA20 and RA40 in 8 hr; RH15 in 12 hr, and RH54 in 16 hr photoperiods. C. giganteum showed significantly more area of seedlings/plantlets under 8 and 12 hr

Fig. 28 : Symbiotic seed germination (%) and seedling growth index of Cymbidium elegans under different photoperiods.

Fig 28

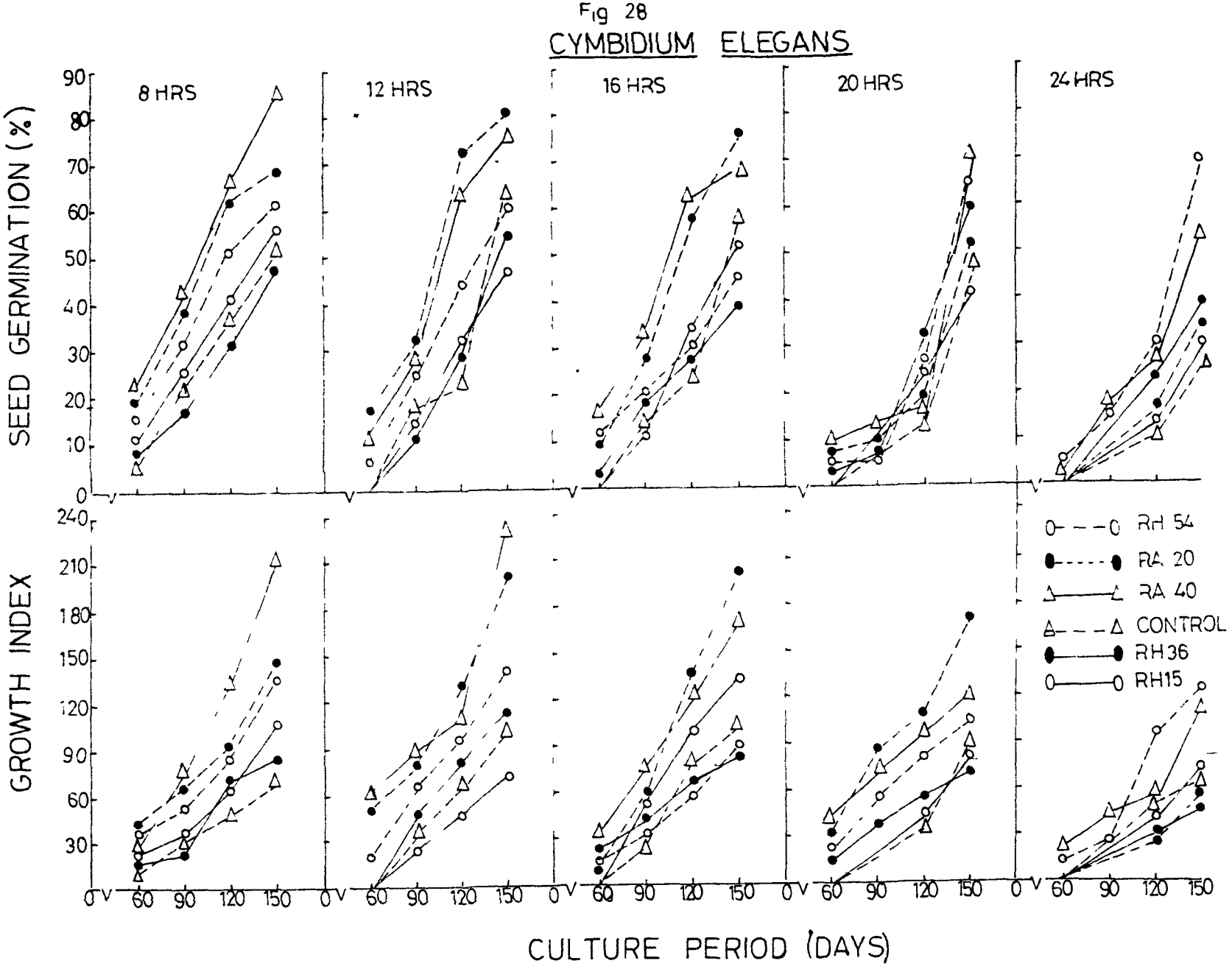

CYMBIDIUM ELEGANS
8 HRS
12 HRS
16 HRS
20 HRS
24 HRS
SEED GERMINATION (%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
RH 54
RA 20
RA 40
CONTROL
RH36
RH15

Fig. 29 : Symbiotic seed germination (%) and seedling growth index of Cymbidium giganteum, under different photoperiods.

Fig: 29

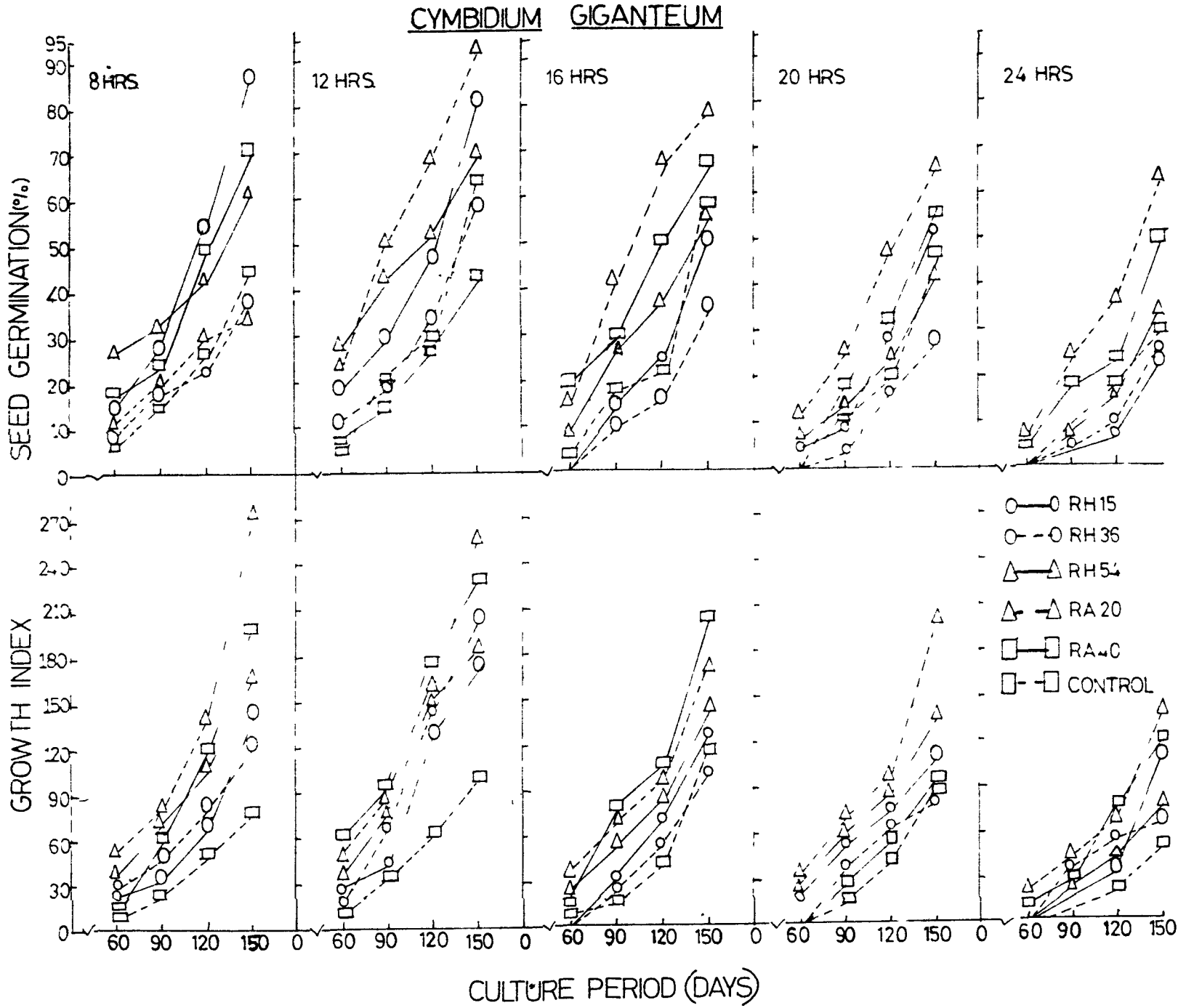

CYMBIDIUM GIGANTEUM
8 HRS
12 HRS
16 HRS
20 HRS
24 HRS
SEED GERMINATION(%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
RH 15
RH 36
RH 54
RA 20
RA 40
CONTROL

Fig. 30 : Symbiotic seed germination (%) and seedling growth index of Thunia alba, under different photoperiods.

Fig. 30.

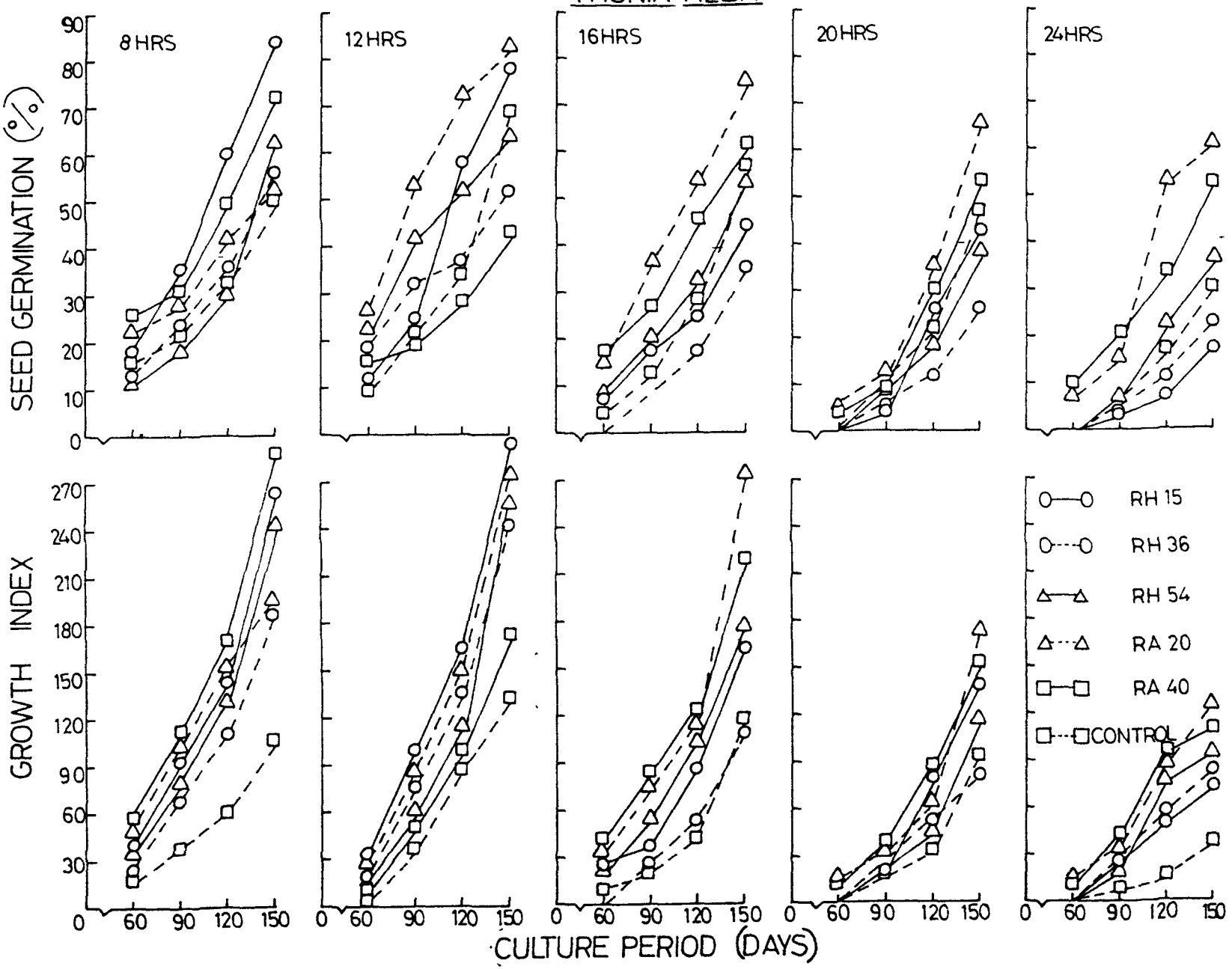

THUNIA ALBA
8 HRS
12 HRS
16 HRS
20 HRS
24 HRS
SEED GERMINATION (%)
GROWTH INDEX
CULTURE PERIOD (DAYS)
RH 15
RH 36
RH 54
RA 20
RA 40
CONTROL

Table 28 : Effect of photoperiods on the growth of seedlings/ plantlets of orchids in symbiotic conditions after 150 days

Orchid species	Fungal isolates	Average area of seedlings/plantlets (mm^2)				
		8 hr	12 hr	16 hr	20 hr	24 hr
C. elegans	RH 15	5.69	9.42	6.49	1.95	2.00
	RH 36	12.00	7.46	10.00	5.66	1.65
	RH 54	7.42	10.80	12.16	2.56	2.32
	RA 20	15.22	12.00	8.99	9.44	5.49
	RA 40	10.00	8.46	9.43	6.22	6.92
	Control	3.49	4.64	2.45	1.66	1.13
C. giganteum	RH 15	5.00	6.42	3.65	2.23	1.98
	RH 36	8.17	12.19	7.96	3.65	2.17
	RH 54	10.50	14.22	13.62	5.79	1.80
	RA 20	18.92	15.76	9.96	8.43	2.79
	RA 40	13.66	7.92	10.00	3.95	1.82
	Control	5.29	4.98	3.66	3.75	2.00
T. alba	RH 15	3.79	5.64	5.11	4.22	2.26
	RH 36	5.96	4.79	10.24	9.23	3.65
	RH 54	8.67	7.99	9.64	5.99	5.96
	RA 20	12.22	8.92	11.62	7.65	8.92
	RA 40	19.22	13.22	12.22	11.15	4.32
	Control	10.95	9.97	6.11	4.76	3.90

Table 29 : Effect of photoperiods on the production of leaf primordia/leaves of orchids in symbiotic conditions after 150 days

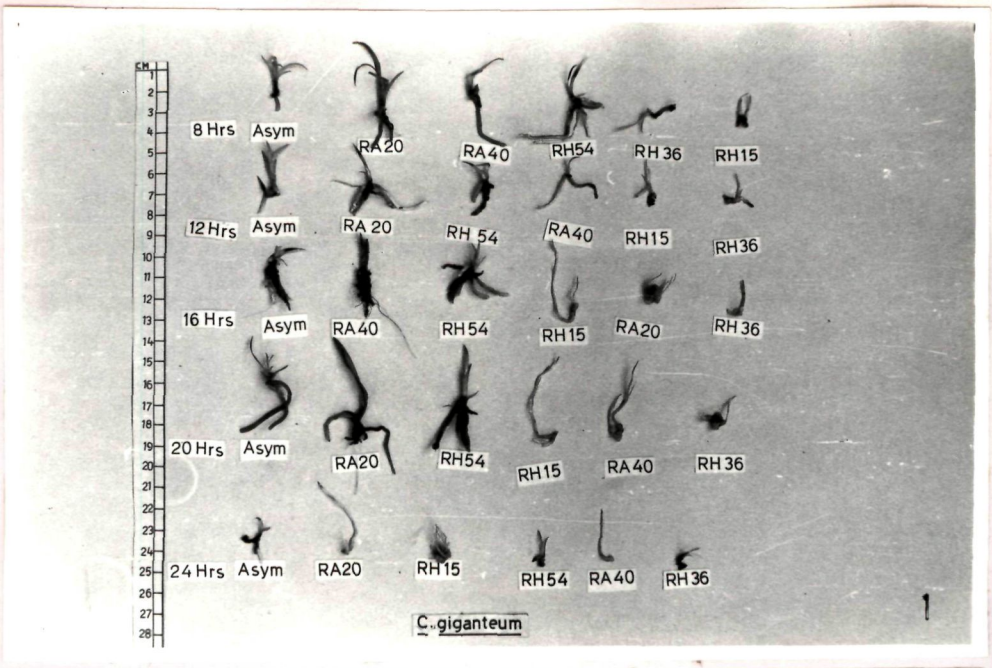
Orchid species	Fungal species	Average number and area of leaf primordia/leaves (mm^2)									
		8 hr		12 hr		16 hr		20 hr		24 hr	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	1.00	0.24	2.11	0.53	1.76	0.40	0.86	0.26	0.75	0.17
	RH 36	2.17	0.79	1.70	0.64	1.54	0.86	1.25	0.33	0.48	0.25
	RH 54	1.15	0.60	2.50	0.78	1.38	0.66	1.00	0.57	0.56	0.47
	RA 20	2.65	1.12	1.82	0.83	2.75	1.08	0.99	0.46	0.91	0.60
	RA 40	2.00	1.05	2.57	0.95	1.35	1.25	0.89	0.50	0.78	0.71
	Control	1.70	0.54	1.88	0.80	1.57	0.67	1.10	0.44	0.50	0.30
C. giganteum	RH 15	1.45	1.26	2.00	1.81	1.15	0.95	0.78	1.17	0.56	0.48
	RH 36	1.67	1.44	2.15	1.06	1.32	1.12	0.80	1.32	0.61	0.76
	RH 54	2.00	1.36	1.00	1.07	1.49	1.22	0.86	1.20	0.71	0.81
	RA 20	2.35	1.49	1.65	0.98	1.58	1.36	1.18	1.29	0.80	0.49
	RA 40	2.66	1.95	1.32	1.69	1.67	1.45	1.10	1.53	0.91	0.55
	Control	1.66	1.48	1.29	1.51	1.25	1.19	0.56	1.29	0.59	0.48
T. alba	RH 15	2.14	3.06	1.60	1.53	1.96	2.00	1.48	0.94	1.18	0.20
	RH 36	1.92	1.25	1.89	1.80	2.45	2.15	1.56	0.45	1.25	0.40
	RH 54	1.96	1.49	1.70	1.75	1.64	1.70	1.32	0.37	1.33	0.35
	RA 20	2.25	2.00	2.56	1.96	2.59	2.59	1.50	0.30	1.59	0.27
	RA 40	1.95	1.55	2.69	1.90	2.15	3.15	1.69	0.46	1.00	0.19
	Control	1.59	1.50	1.86	1.40	1.79	1.70	1.32	0.47	1.20	0.22

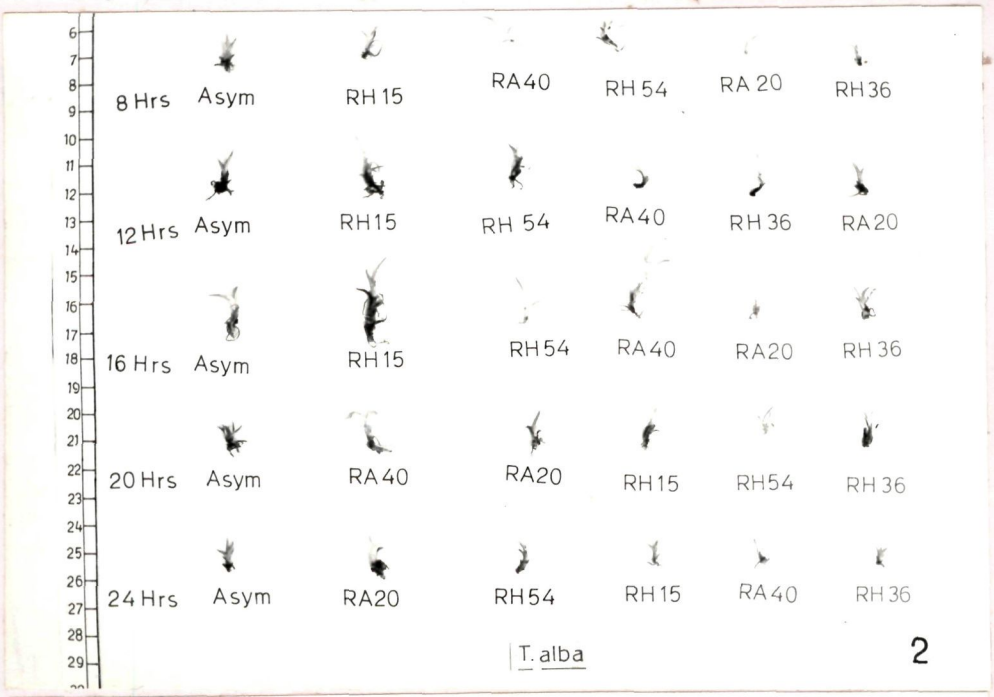
Table 30 : Effect of photoperiods on the production of rhizoids/roots of orchids in symbiotic conditions after 150 days

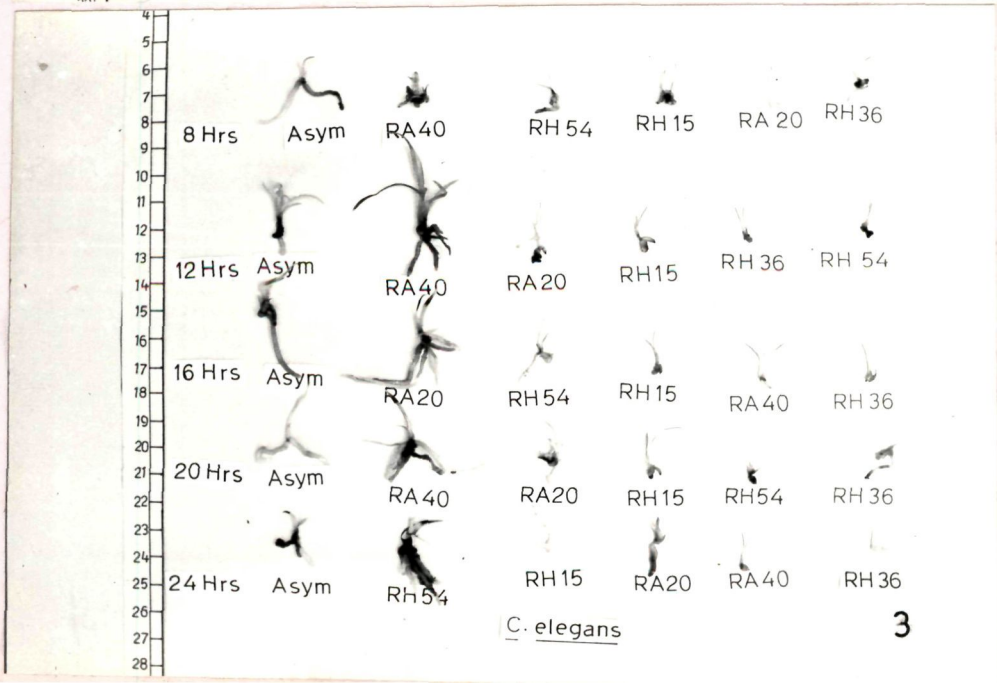
Orchid species	Fungal isolates	Average number and area of rhizoids/roots (mm^2)									
		8 hr		12 hr		16 hr		20 hr		24 hr	
		No.	area	No.	area	No.	area	No.	area	No.	area
C. elegans	RH 15	6.79	0.18	9.42	0.26	8.19	0.09	3.10	0.02	2.29	0.13
	RH 36	8.49	0.21	5.32	0.31	3.45	0.11	8.12	0.14	1.96	0.07
	RH 54	10.22	0.19	4.46	0.15	11.39	0.08	7.00	0.09	7.22	0.02
	RA 20	7.98	0.20	8.55	0.18	13.22	0.17	4.22	0.16	8.91	0.09
	RA 40	9.67	0.15	4.49	0.16	10.26	0.06	5.92	0.15	9.55	0.15
	Control	6.92	0.12	7.98	0.10	5.11	0.04	5.75	0.02	3.48	0.06
C. giganteum	RH 15	7.42	0.17	5.72	0.56	4.95	0.62	2.79	0.47	1.54	0.15
	RH 36	10.92	0.35	6.45	0.28	3.86	0.33	6.45	0.15	2.96	0.32
	RH 54	10.00	1.00	9.67	0.96	6.92	1.23	8.96	0.65	3.82	0.41
	RA 20	13.15	1.25	11.00	1.05	8.75	0.95	7.27	0.74	4.96	0.13
	RA 40	8.45	0.22	7.84	1.22	6.22	0.65	5.82	0.49	5.00	0.20
	Control	9.66	0.18	6.22	0.34	4.96	0.30	3.33	0.21	2.96	0.25
	RH 15	1.10	0.21	2.15	0.16	1.16	0.26	1.00	0.20	0.66	0.14
	RH 36	1.25	0.45	1.25	0.14	1.25	0.45	1.25	0.14	0.96	0.20
	RH 54	2.00	0.61	1.32	0.25	2.19	0.30	1.68	0.25	1.29	0.35
	RA 20	1.35	0.32	1.41	0.64	1.06	0.65	1.28	0.40	1.00	0.11
	RA 40	1.49	0.49	2.65	0.88	1.54	0.49	1.32	0.29	0.88	0.15
	Control	1.04	0.25	1.21	0.19	1.82	0.33	1.11	0.20	0.80	0.19

Plate 14 : Symbiotic seedling growth of *C. giganteum* (Fig. 1), *T. alba* (Fig. 2) and *C. elegans* (Fig. 3), under different photoperiods.

PLATE 14


8 Hrs Asym
RA20
RA40
RH54
RH36
RH15
12 Hrs Asym
RA20
RH54
RA40
RH15
RH36
16 Hrs Asym
RA40
RH54
RH15
RA20
RH36
20 Hrs Asym
RA20
RH54
RH15
RA40
RH36
24 Hrs Asym
RA20
RH15
RH54
RA40
RH36
C. giganteum
1


8 Hrs Asym
RH15
RA40
RH54
RA20
RH36
12 Hrs Asym
RH15
RH54
RA40
RH36
RA20
16 Hrs Asym
RH15
RH54
RA40
RA20
RH36
20 Hrs Asym
RA40
RA20
RH15
RH54
RH36
24 Hrs Asym
RA20
RH54
RH15
RA40
RH36
T. alba
2


8 Hrs Asym
RA40
RH54
RH15
RA20
RH36
12 Hrs Asym
RA40
RA20
RH15
RH36
RH54
16 Hrs Asym
RA20
RH54
RH15
RA40
RH36
20 Hrs Asym
RA40
RA20
RH15
RH54
RH36
24 Hrs Asym
Rh54
RH15
RA20
RA40
RH36
C. elegans
3

photoperiods than the other photoperiods (Table 28). Significantly higher area of seedlings/plantlets in T. alba was observed at 8 hr photoperiod using RA40 followed by RA20, RH15 at 12 hr, RH36 and RH54 at 16 hr photoperiods (Table 28). Statistically significant variations were obtained in relation to the seedling growth at 1% level between different photoperiods and 5% level between fungal isolates, in all the three species.

Significantly, more leaf primordia/leaves in C. elegans were produced using RH36 in 8 hr; RH15, RH54 and RA40 in 12 hr and RA20 in 16 hr photoperiods (Table 29). Whereas, comparatively higher number and area of leaf primordia/leaves in C. giganteum were induced using RH54, RA20 and RA40 in 8 hr, and using RH15 and RH36 in 12 hr photoperiods (Table 29). Average number of area of leaf primordia/leaves in T. alba was better under 8 hr and 16 hr photoperiods than the other photoperiods with all the mycorrhizal fungi (Table 29).

The rhizoids/roots were higher in seedlings of C. elegans inoculated with RH36, RH54 and RA40 in 8 hr, with RH15 at 12 hr and with RA20 in 16 hr photoperiods. Whereas, significantly more number and area of rhizoids/roots were produced in C. giganteum using RH36, RH54 and RA40 in 8 hr and using RH15 and RA20 in 12 hr photoperiods (Table 30).

Thunia alba exhibited the maximum number and area of rhizoids/ roots with RH36 at 8 hr, with RH15, RA20 and RA40 at 12 hr and with RH54 at 16 hr photoperiod. The lowest development of rhizoids/roots was seen under 12 hr photoperiod in all the cases (Table 30).

III. Growth Hormones Production by Mycorrhizal Fungi of Orchids

1) Qualitative analyses of auxins, cytokinins and gibberellins

a) Auxins

The qualitative analysis of auxins indicates that a total of five auxins were detected from mycorrhizal fungi (Table 31; Fig. 31). IAA, IBA, IAN and $IPyA$ were detected in RH51 fungal isolate in ten days old culture whereas, RH46 and RH54 endophytes produced only IAA after ten days. RH36 isolate, however, could produce only two auxins at the age of sixty days (Table 31). From Fig. 31 it is clear that the number of auxins varied with age of the culture medium. The higher number of auxins were detected in fungal extract of RH51, RH46 and RH61 endophytes than in the other mycorrhizal fungi (Table 31).

b) Cytokinins

Table 32 shows that three cytokinins were detected in the fungal extracts of culture medium. Kinetin

Fig. 31 : Number of auxins, cytokinins and gibberellins produced by different mycorrhizal fungi of the orchids in pure culture.

Fig. 31

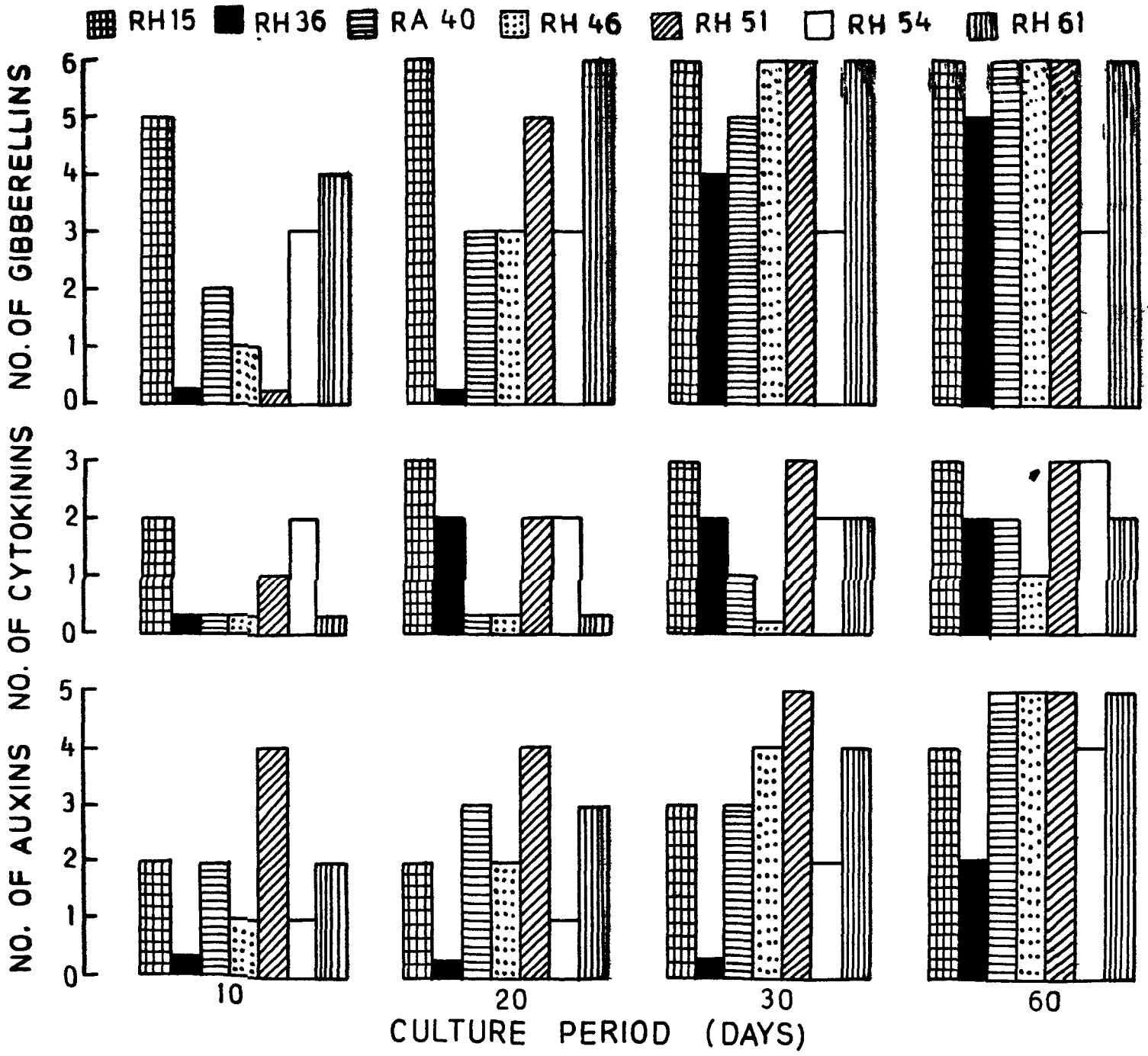

RH 15
RH 36
RA 40
RH 46
RH 51
RH 54
RH 61
NO. OF GIBBERELLINS
NO. OF CYTOKININS
NO. OF AUXINS
0
1
2
3
4
5
6
10
20
30
60
CULTURE PERIOD (DAYS)

Table 31 : Distribution pattern of auxins in mycorrhizal fungi of orchids in pure culture

Fungal isolates	Culture period (days)																			
	10					20					30					60				
	IAA	IBA	IGA	IAN	IPYA	IAA	IBA	IGA	IAN	IPYA	IAA	IBA	IGA	IAN	IPYA	IAA	IBA	IGA	IAN	IPYA
RH 15	+	-	-	+	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+
RH 36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
RA 40	+	-	-	+	-	+	+	-	+	-	+	+	-	+	-	+	+	+	+	+
RH 46	+	-	-	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+	+
RH 51	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
RH 54	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+
RH 61	+	-	-	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+

IAA = Indoleacetic acid; IBA = Indolebutyric acid; IGA = Indoleglycolic acid; IAN = Indoleacetonitrite;

IPYA = *Indolepyruvic acid*

\+ = Present

\- = Absent.

Table 32 : Distribution pattern of cytokinins in mycorrhizal fungi of orchids in pure culture

Fungal isolates	Culture period (days)											
	10			20			30			60		
	KI	2ip	UNC	KI	2ip	UNC	KI	2ip	UNC	KI	2ip	UNC
RH 15	+	+	-	+	+	+	+	+	+	+	+	+
RH 36	-	-	-	-	+	+	-	+	+	-	+	+
RA 40	-	-	-	-	-	-	-	-	+	+	-	+
RH 46	-	-	-	-	-	-	-	-	-	-	-	+
RH 51	+	-	-	+	-	+	+	+	+	+	+	+
RH 54	-	+	+	+	+	-	+	+	-	+	+	-
RH 61	-	-	-	-	-	-	-	+	+	-	+	+

KI = Kinetin; UNC = Unidentified compound; 2ip = N-6 (- isopentenyl) adenine;

\+ = Present

\- = Absent

Table 33 : Distribution pattern of gibberellins in mycorrhizal fungi of orchids in pure culture

Fungal isolates	Culture period (days)											
	10			20			30			60		
	GA 2,4,9	GA 3,7	GA 5	GA 2,4,9	GA 3,7	GA 5	GA 2,4,9	GA 3,7	GA 5	GA 2,4,9	GA 3,7	GA 5
RH 15	+	+	-	+	+	+	+	+	+	+	+	+
RH 36	-	-	-	-	-	-	+	-	+	+	+	-
RA 40	-	+	-	-	+	+	+	+	-	+	+	+
RH 46	-	-	+	-	+	+	+	+	+	+	+	+
RH 51	-	-	-	+	+	-	+	+	+	+	+	+
RH 54	-	+	+	-	+	+	-	+	+	-	+	+
RH 61	+	-	+	+	+	+	+	+	+	+	+	+

GA = Gibberellic acid

\+ = Present

\- = Absent

and 2ip were detected only in the RH15 strain after ten days of culture. While, in RA40, RH46 and RH61 no cytokinin was detected even upto twenty days of culture (Table 32), RH54 contained 2ip and an unidentified compound on 10th day of culture. It is clear that number of cytokinins varied in different mycorrhizal fungi with different ages of culture and more number of cytokinins were detected in RH15, and RH51 than in the other fungal isolates.

c) Gibberellins

The qualitative analysis of gibberellins of mycorrhizal fungi indicates that a total of six gibberellins were detected (Table 33; Fig. 31). Five GA_s were produced in RH15 isolate whereas, RH51 and RH36 showed no gibberellins in 10 day-old culture (Table 33). The number of gibberellins increased with fungal culture-age.

2) Quantitative determination of total auxins, cytokinins and gibberellins

a) Auxins

The quantity of total auxins varied in different fungal isolates at different culture periods (Fig. 32). The amount of total auxins increase with age of the fungal culture and maximum amount was in RH51 followed by RH61 and RH46 isolates. The lowest quantity was recorded in case of RH36 endophyte.

Fig. 32 : Quantity of total auxins produced by different mycorrhizal fungi of the orchids in pure culture.

Fig.32

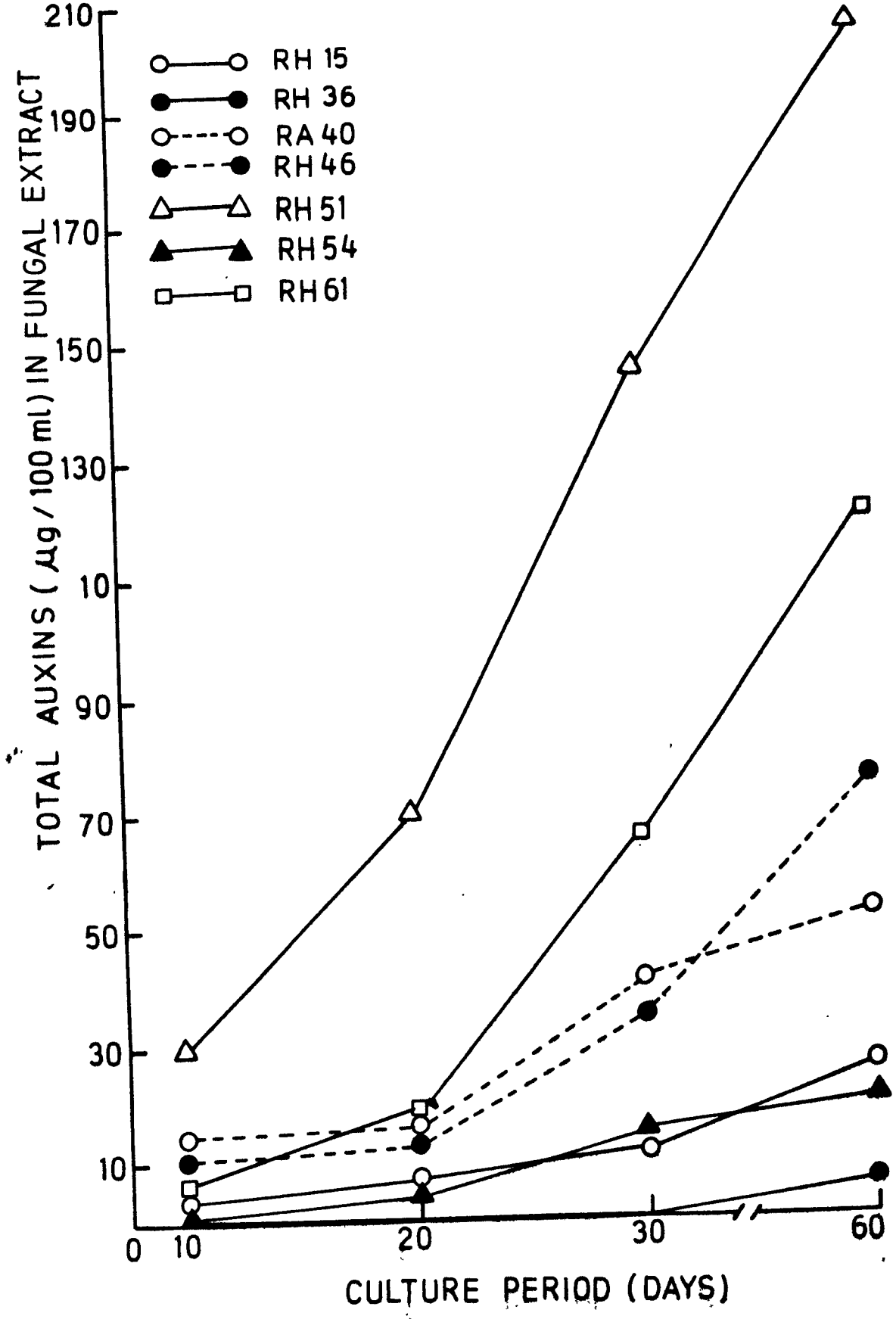
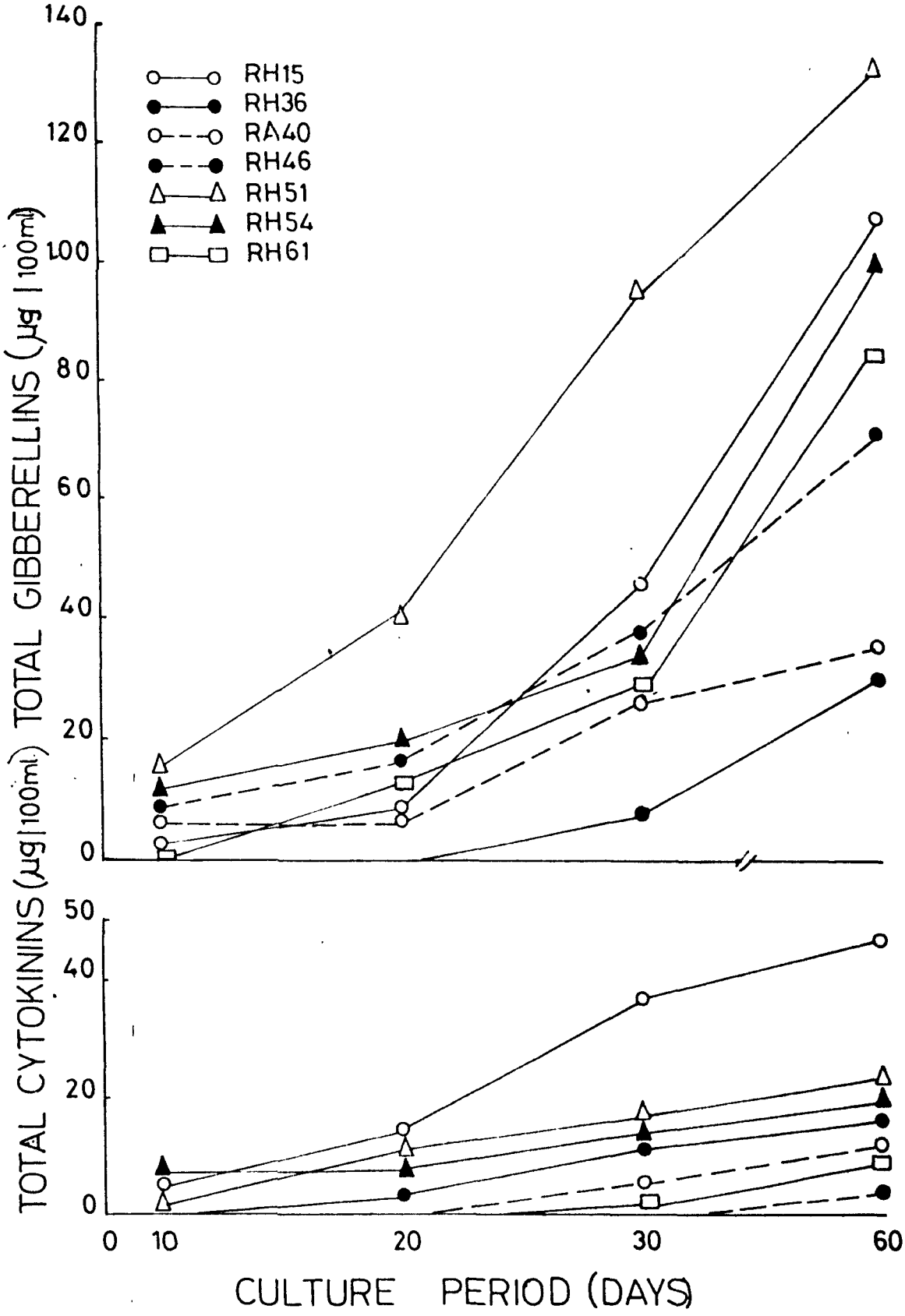

RH 15
RH 36
RA 40
RH 46
RH 51
RH 54
RH 61
TOTAL AUXINS (μg/100ml) IN FUNGAL EXTRACT
210
190
170
150
130
10
90
70
50
30
10
0
10
20
30
60
CULTURE PERIOD (DAYS)

Fig. 33 : Quantity of total cytokinins and gibberellins produced by the different mycorrhizal fungi of the orchids in pure culture.

Fig.33


RH15
RH36
RA40
RH46
RH51
RH54
RH61
TOTAL GIBBERELLINS (μg / 100ml)
TOTAL CYTOKININS (μg/100ml)
140
120
100
80
60
40
20
0
50
40
20
0
0
10
20
30
60
CULTURE PERIOD (DAYS)

b) Cytokinins

The amount of total cytokinins also differed in different mycorrhizal fungi at various culture periods (Fig. 33). The quantity of total cytokinins increased with the age of the culture. The highest amount was noticed in RH15 followed by RH51 strain. The lowest quantity was obtained in RH46 isolate.

c) Gibberellins

The quantity of total gibberellins was enhanced with the age of the cultures of the mycorrhizal fungi studied (Fig. 33). The amount of total GA_s was initially higher in RH51 isolate than in the other six fungal endophytes. While it was maximum in RH51 and in RH36 the amount was least at the age of sixty days (Fig. 33).

IV. Phosphorus uptake and Phosphatase Activity in Mycorrhizal Seedlings of Orchids

1) Phosphorus uptake

The growth of mycorrhizal and non-mycorrhizal seedlings of orchids in the earthen pots varied with the age of the seedlings at different phosphate levels (Table 34). Better dry weight of seedlings was observed at lower and medium doses of phosphate in the mycorrhizal sets compared to the non-mycorrhizal ones in both the cymbidium species.

Table 34 : Dry weight of mycorrhizal and non-mycorrhizal seedlings (g/pot), grown at different phosphate levels

Orchid species	Phosphate levels (mg/pot)		Harvesting periods (days)		
			30	60	90
C. elegans	0.0	+M	0.08*	0.09*	0.10
		-M	0.05	0.06	0.09
	1.68	+M	0.05	0.12*	0.17
		-M	0.04	0.05	0.15
	3.36	+M	0.06	0.14*	0.29*
		-M	0.05	0.06	0.18
	33.6	+M	0.10	0.10	0.20
		-M	0.10	0.08	0.19
	67.2	+M	0.06*	0.09	0.18
		-M	0.01	0.09	0.20
C. giganteum	0.0	+M	0.06	0.07	0.12*
		-M	0.04	0.06	0.09
	1.68	+M	0.07	0.09	0.14*
		-M	0.06	0.08	0.
	3.36	+M	0.09	0.15*	0.20*
		-M	0.07	0.10	0.13
	33.6	+M	0.18*	0.23*	0.26*
		-M	0.09	0.14	0.17
	67.2	+M	0.08	0.16	0.19
		-M	0.10	0.17	0.22*

+M = Mycorrhizal; -M = Non-mycorrhizal; * = Significant.

Fig. 34 : Percentage phosphorus content in mycorrhizal and non-mycorrhizal seedlings of C. elegans, grown in the soil mixtures containing different phosphate levels.

Fig.34

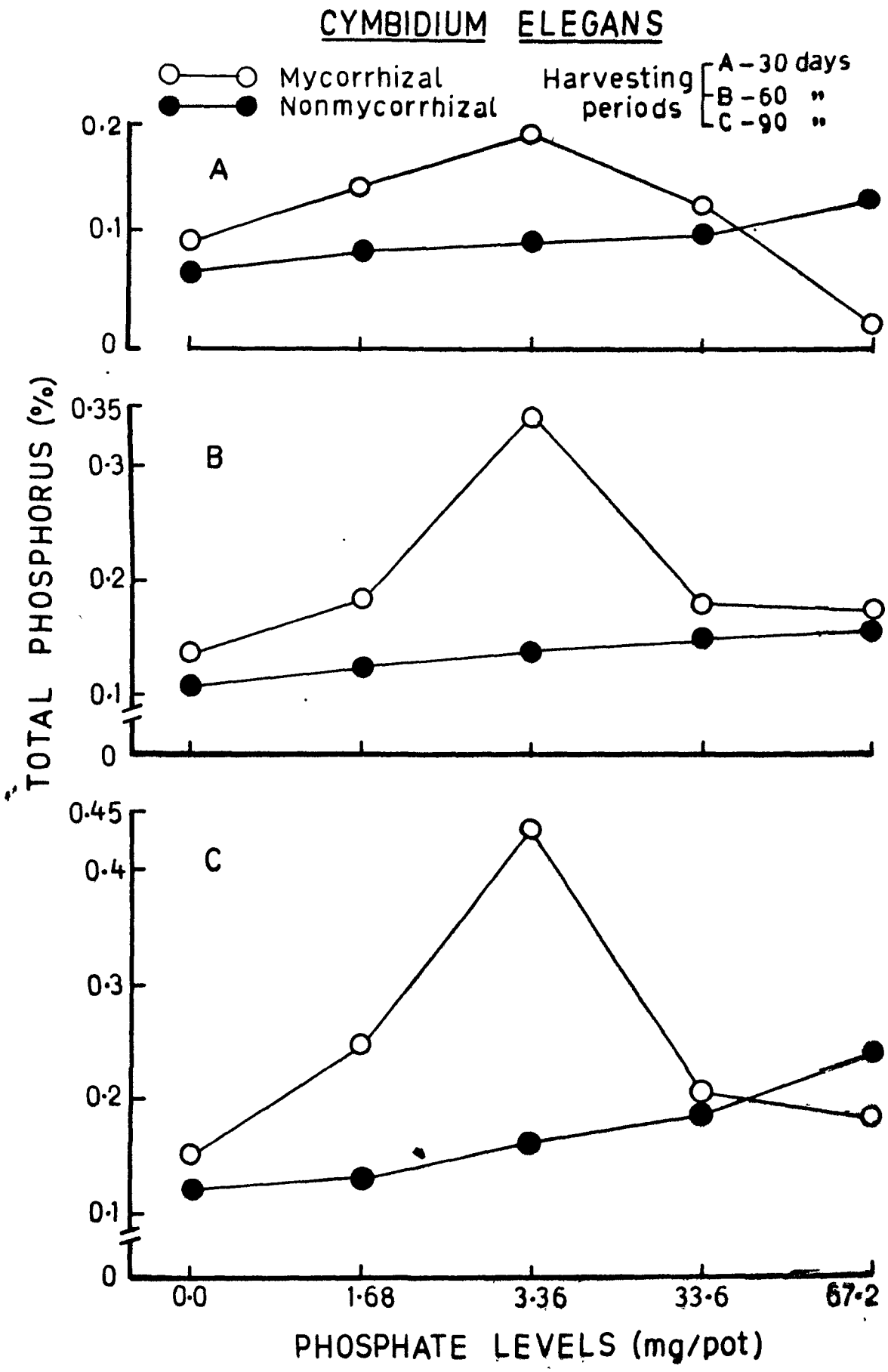
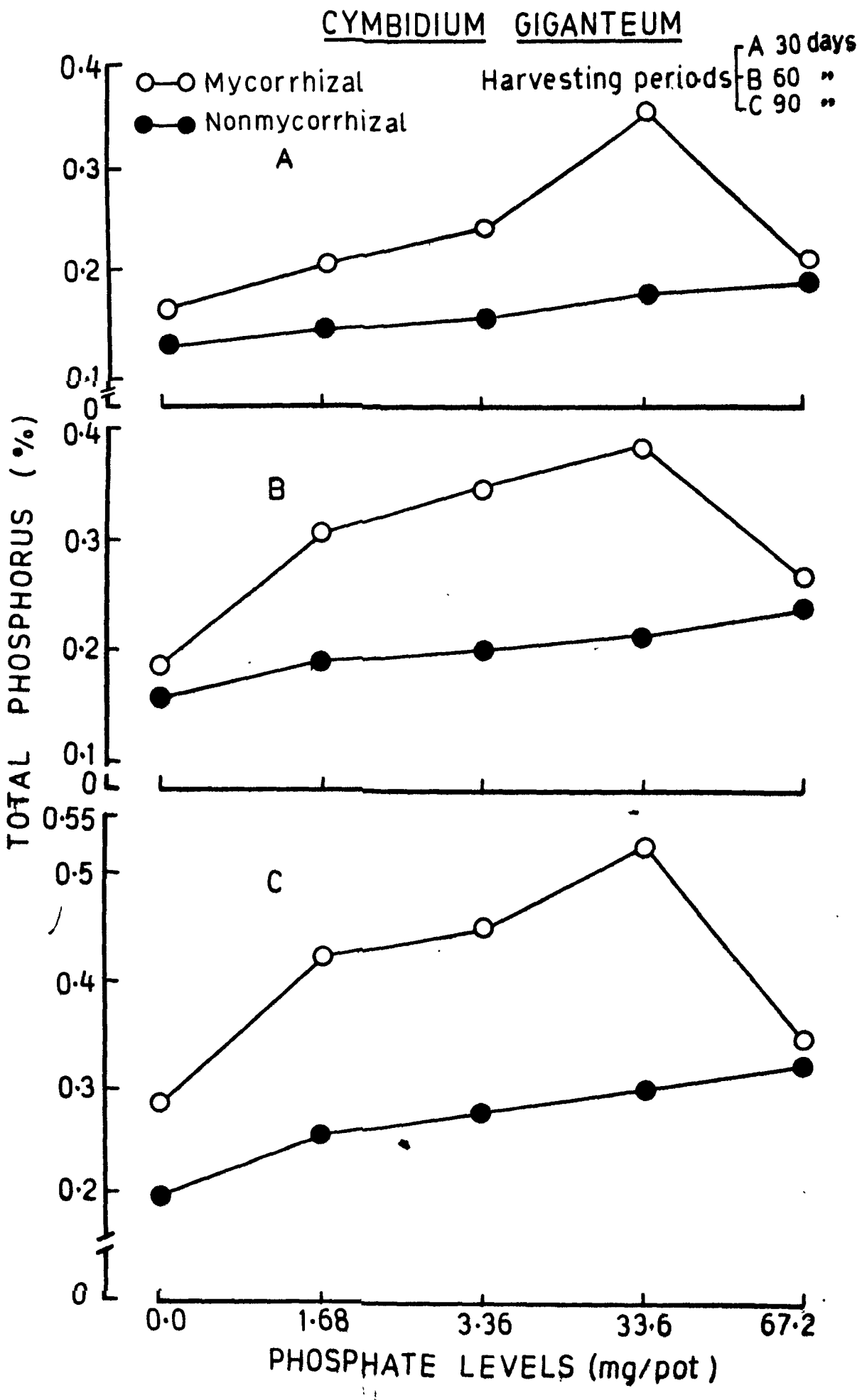

CYMBIDIUM ELEGANS
Mycorrhizal
Nonmycorrhizal
Harvesting periods
A – 30 days
B – 60 "
C – 90 "
A
B
C
0·2
0·1
0
0·35
0·3
0·2
0·1
0
0·45
0·4
0·3
0·2
0·1
0
TOTAL PHOSPHORUS (%)
0·0
1·68
3·36
33·6
67·2
PHOSPHATE LEVELS (mg/pot)

Fig. 35 : Percentage phosphorus content in mycorrhizal and non-mycorrhizal seedlings of C. giganteum, grown in the soil mixtures containing different phosphate levels.

Fig.35


CYMBIDIUM GIGANTEUM
Mycorrhizal
Nonmycorrhizal
Harvesting periods
A 30 days
B 60 "
C 90 "
A
B
C
TOTAL PHOSPHORUS (%)
0·4
0·3
0·2
0·1
0
0·55
0·5
0·4
0·3
0·2
0
0·0
1·68
3·36
33·6
67·2
PHOSPHATE LEVELS (mg/pot)

The dry weight of mycorrhizal seedlings significantly increased with the increase of phosphate application upto 3.36 mg/pot in C. elegans and upto 33,6 mg/pot in case of C. giganteum, and then decreased abruptly with further increase in the phosphate levels. These increases were more pronounced at 60 and 90 days of growth. No significant variations in dry weight were observed between inoculated and uninoculated seedlings at higher phosphate levels (Table 34).

The non-mycorrhizal seedlings in C. giganteum showed increasing trend with the increase of phosphate levels and they were significantly different from mycorrhizal seedlings at the highest phosphate level (Table 34).

The phosphorus content of mycorrhizal seedlings of C. elegans significantly increased upto 3.36 mg/pot phosphate level and then showed a decreasing trend. But the phosphorus content of non-mycorrhizal seedlings increased with the increase in the phosphate levels (Fig. 34). Whereas, C. giganteum showed increasing trend of phosphorus content upto 33.6 mg/pot phosphate level and then declined at higher phosphate level (Fig. 35).

2) Phosphatase activity

The fresh weight of mycorrhizal and non-mycorrhizal seedlings of orchids also differed with respect to varying phosphate additions in soil mixture.

Table 35 : Fresh weight of mycorrhizal and non-mycorrhizal seedlings (g/pot) of Cymbidium elegans grown at different phosphate levels

Phosphate levels (mg/pot)		Harvesting periods (days)		
		30	60	90
0.0	+M	0.86*	1.14*	1.26*
	-M	0.44	0.75	0.95
1.68	+M	0.20*	1.40*	1.58
	-M	0.76	0.96	1.43
3.36	+M	1.50*	1.66*	1.80*
	-M	0.95	1.10	1.35
33.6	+M	1.15	1.46	1.60
	-M	1.25*	1.38	1.65
67.2	+M	0.70	0.90	1.04
	-M	0.50	0.69	1.20

+M = Mycorrhizal; -M = Non-mycorrhizal; * = Significant.

Table 36 : Fresh weight of mycorrhizal and non-mycorrhizal seedlings (g/pot) of Cymbidium giganteum grown at different phosphate levels

Phosphate levels (mg/pot)		Harvesting periods (days)		
		30	60	90
0.0	+M	1.12*	1.26*	1.54*
	-M	0.56	0.71	0.96
1.68	+M	1.60*	1.81*	1.92*
	-M	0.96	1.15	1.36
3.36	+M	1.00	1.30	1.55
	-M	1.25*	1.44	1.78
33.6	+M	0.90	1.20	1.49
	-M	1.46*	1.58*	1.88*
67.2	+M	0.67	0.95	1.27
	-M	0.70	1.12	1.50*

+M = Mycorrhizal; -M = Non-mycorrhizal; * = Significant.

Plate 15 : Growth of mycorrhizal and non-mycorrhizal seedlings of *Cymbidium giganteum* (Fig. 1) and *C. elegans* (Fig. 2), in the earthen pots.

PLATE 15

1

2

The fresh weight of seedlings significantly increased with the advanced age of the seedlings (Table 35 and 36). Significantly, higher fresh weight was obtained upto 3.36 mg/pot phosphate level in the inoculated seedlings than the uninoculated ones in C. elegans. Whereas, mycorrhizal seedlings of C. giganteum exhibited significantly better fresh weight upto 1.68 mg/pot phosphate level and then decreased abruptly with further increase in the phosphate level (Table (Table 36).

In case of non-mycorrhizal seedlings, the fresh weight increased with the increase in the phosphate application upto 33.6 mg/pot phosphate level and then decreased in both the species (Tables 35 and 36).

The acid and alkaline phosphatase activities of orchid seedlings also varied with the age of seedlings at different phosphate levels (Figs. 36 and 37). Initially, at 30 days of growth both the acid and alkaline phosphatase activities were significantly higher and then decreased with the increase of seedlings age in both the species. At lower phosphate levels, the acid phosphatase activity was higher but it decreased at higher phosphate levels (Figs. 36 and 37). The uninoculated seedlings showed more alkaline phosphatase activity than the inoculated ones in the control sets (without phosphate additions in the soil mixture). Conversely, the

Fig. 36 : Acid and alkaline phosphatase activities in mycorrhizal and non-mycorrhizal seedlings of C. elegans grown in the soil mixtures containing different phosphate levels.

Fig. 36

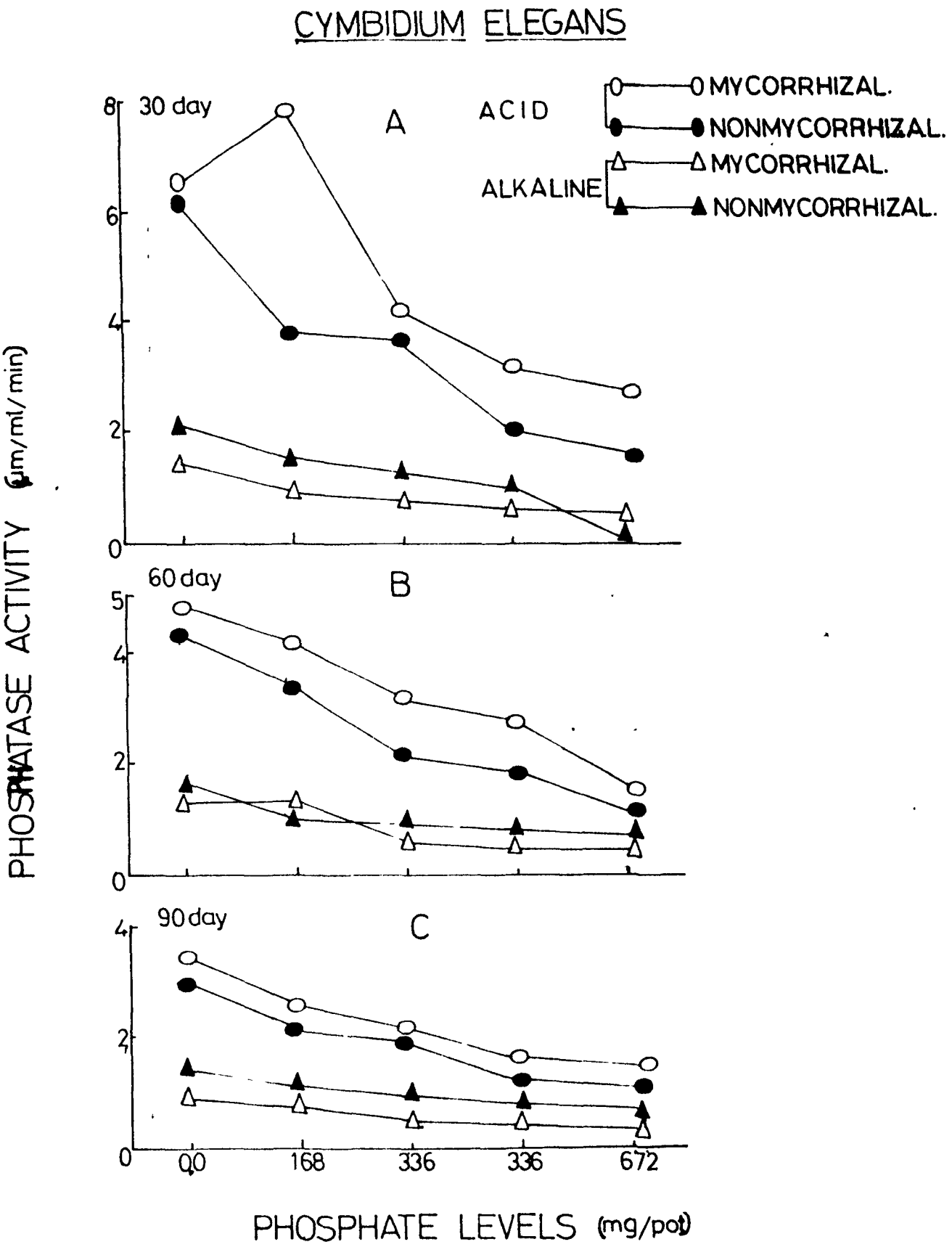
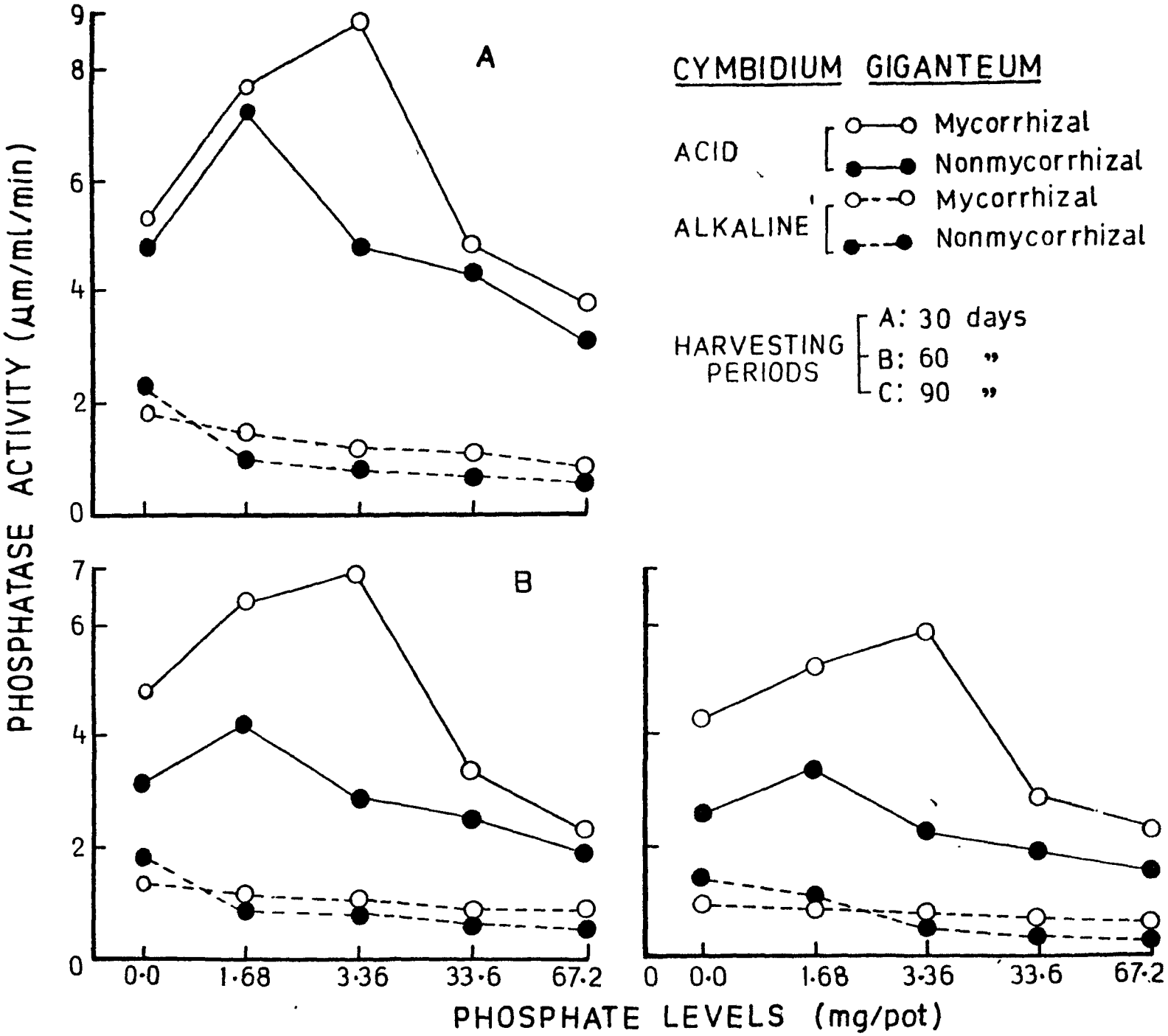

CYMBIDIUM ELEGANS
30 day
A
ACID
MYCORRHIZAL.
NONMYCORRHIZAL.
ALKALINE
MYCORRHIZAL.
NONMYCORRHIZAL.
60 day
B
90 day
C
PHOSPHATASE ACTIVITY (μm/ml/min)
0
2
4
6
8
5
0
168
336
336
672
PHOSPHATE LEVELS (mg/pot)

Fig. 37 : Acid and alkaline phosphatase activities in mycorrhizal and non-mycorrhizal seedlings of C. giganteum grown in the soil mixtures containing different phosphate levels.

Fig. 37


CYMBIDIUM GIGANTEUM
ACID
Mycorrhizal
Nonmycorrhizal
ALKALINE
Mycorrhizal
Nonmycorrhizal
HARVESTING PERIODS
A: 30 days
B: 60 "
C: 90 "
A
B
PHOSPHATASE ACTIVITY (μm/ml/min)
0
2
4
6
8
9
7
0·0
1·68
3·36
33·6
67·2
PHOSPHATE LEVELS (mg/pot)

mycorrhizal seedlings exhibited significantly higher acid phosphatase activity than the non-mycorrhizal seedlings in controls of both the species. The acid and alkaline phosphatase activities decreased in both the mycorrhizal and non-mycorrhizal seedlings with the increase of phosphate levels in the soil (Figs. 36 and 37).

DISCUSSION.

In a vast majority of the cultivated orchids, the flowers are not pollinated, as a consequence of which, capsules are rarely formed. Even when seeds are set, less than 5% of them germinate under natural conditions because of their particular fungal requirement (Mathews *et al.*, 1980). During germination high mortality is observed at the protocorm stage both *in vitro* and *in vivo* conditions (Arditti *et al.*, 1981). The mycorrhizal association helps in protocorm development and seedling establishment in natural conditions (Meyer, 1966; Patrick *et al.*, 1980; Terashita, 1985) on account of its role in nutrient uptake from substrate to germinating protocorms (Hadley, 1984; Alexander and Hadley, 1985). Therefore, to maintain the existing population and multiplication of rare and endangered orchids on large scale, the higher and faster seed germination of orchids *in vitro* could be obtained on enriched nutrient medium under suitable temperature and light conditions (Harvais, 1982; Nakamura, 1982; Miura, 1982; Zeiger *et al.*, 1985). Further seedling growth is reported to be greatly enhanced in symbiotic conditions rather than in asymbiotic conditions.

Significant stimulation in asymbiotic seed germination, seedling growth, root and leaf number and area was observed at 20°C temperature in case of *C. elegans*, whereas in *C. giganteum* and *T. alba*, these were higher at 25°C and

30°C, respectively (Figs. 3 and 4, Tables 1-3 and Plate 5).

The symbiotic seed germination and seedling development of orchids were also higher at lower to moderate temperatures (20-30°C; Figs. 13-18, Tables 16-18 and Plate 10). However, higher temperatures were inhibitory. The higher seed germination and subsequent seedling development in all the species were recorded in symbiotic conditions compared to respective controls. Both in asymbiotic and symbiotic conditions, maximum death of protocorms/plantlets and shrinkage of the medium occurred at 35°C temperature.

In the present studies the higher seed germination and overall seedling growth of orchids were recorded in asymbiotic conditions (on enriched nutrient medium, i.e., modified Kn C medium) than in the symbiotic conditions (on poor nutrient medium, i.e., Oat medium). Knudson (1925) suggested that fungus is not necessarily required for seed germination, if the synthetic medium is supplemented with various substances like vitamins, amino acids, and growth hormones, etc. Many workers have reported good seed germination and seedling development asymbiotically on enriched nutrient media in different orchids (Zeigler et al., 1967; Ueda and Torikata, 1972; Mead and Bulard, 1975; Linden, 1980; Arditti et al., 1981; Raghuwanshi et al., 1985).

It is generally believed that in mycorrhizal association the symbiotic fungus stimulates both germination and growth of orchids. There are many orchid species in which the seeds will not germinate at all in the absence of suitable fungus (Harley, 1959). This was not so with Orchis purpurella seeds which germinated readily by themselves in culture. The seedlings obtained from symbiotic cultures are strong, adapt easily and establish readily in pot-soil culture. It seems that the symbionts also provide some protection to the orchid seedlings from other soil pathogens (Clements et al., 1979).

Bernard (1903) noticed better symbiotic germination and seedling growth in Cattleya mossiae and Lealia purpurea orchids at 28°C temperature. In case of Cymbidium good asymbiotic germination and growth within the range of 21-29°C temperature was reported by Scott and Arditti, 1959). The better results obtained for C. elegans, C. giganteum and T. alba during the present investigations also fall well within this temperature range. Increased germination and growth of seedlings of these species between the range of 20-30°C temperature was expected because of higher plant metabolic rate within this temperature range (Knudson, 1950; Miura, 1982; Zeiger et al., 1985). Biddington et al. (1980) showed that modrate temperature does not increase metabolic activity directly but makes the embryonic tissue more

sensitive to water and nutrient absorption from the medium. The inhibition in seed germination and seedling growth at extreme temperatures (around 35°C) may be due to the loss of water from the medium as well as from the living system (Zeigler et al., 1967).

The improved symbiotic germination and growth of orchids at lower to moderate temperatures could be due to balanced association between the symbiotic fungal strains and the protocorms (Harvais et al., 1967; Clements et al., 1979; Warcup, 1980). Mycorrhizal seedlings showed higher growth than the controls at all the temperatures studied. This could be due to easy mobilization of complex nutrients in protocorms through the mycorrhizal fungi (Hadley, 1984; Alexander and Hadley, 1985). On the other hand, slow growth of the asymbiotic protocorms may be due to the presence of excess starch (Purves and Hadley, 1976). Certain fungal symbionts considerably reduced the germination and growth of seedlings at extreme temperatures on account of imbalanced symbiotic relationship, which ultimately resulted in parasitism (Williamson and Hadley, 1970; Hadley, 1983; Alexander and Hadley, 1983).

The asymbiotic seed germination and seedling development was significantly higher between pH 4.0 - 6.0 in all the species (Figs. 5 and 6; Tables 4-6 and Plate 6). Cymbidium

elegans, exhibited more seed germination at pH 3.0 and 4.0 under symbiotic condition than at the other pH values (Fig. 19). On the other hand, in inoculated cultures, pH 5.0 and 6.0 were better for germination in C. giganteum and T. alba, respectively (Figs. 20 and 21). Likewise, growth index, area of seedlings/plantlets, number and area of roots and leaves were also enhanced between pH 4.0 - 6.0 under symbiotic conditions in all the species (Fig. 19-21 and Tables 19-21). However, both seed germination and seedling growth were markedly inhibited at pH 10.0.

Cappelletti (1933) noticed higher germination of Cymbidium seeds in the pH range of 3.0 - 6.0. In Dendrobium species, within the stimulatory pH range of 4.0 - 5.4, pH 4.6 was found optimum (Kotomori and Murashige, 1965). Knudson (1946a) observed the best seed germination at pH 5.0 in the case of Cattleya mossiae. Goh (1971) studied the effect of pH on the absorption of phosphate by the terrestrial roots of two orchid hybrids and observed most efficient uptake in the range of pH 5.0 to 5.5. He suggested that this range of pH might also be suitable for maximum absorption of water and nutrients from the medium. On the contrary, during the present studies, the seed germination and subsequent seedling growth of orchids decreased at extreme pH values, i.e., pH 3.0 and 10.0.

Hewitt (1966) studied the influence of pH on the growth of plants like tomato, lettuce, citrus, barley, wheat, maize, etc. and suggested that these plants can tolerate pH values from 4.0 - 8.0. However, at lower values of 3.5 - 4.0, or at higher values 8.0 - 8.5, marked depression of growth occurs and at more extreme pH, injury of tissues results (Stephen and Chan, 1970). The low pH conditions may also affect the synthesis process by altering the enzymatic activities in the cell (Whitaker, 1976).

The majority of efficient nutrient solutions have pH values between 5.0 and 6.0 and these limits are associated with healthy growth of many plants (Goh, 1971). At pH 4.0 or less, uptake of potassium and calcium may be depressed and at higher pH values, precipitation and non-utilization of iron compounds may occur (Hewitt, 1966).

The enhanced symbiotic seed germination and seedling growth of orchids in slightly acidic pH (pH 4.0 - 6.0) of the medium was expected due to increased uptake of nutrients (Kotomori et al., 1965; Landecker, 1972; Withner, 1974; Paleman et al., 1976) and balanced symbiotic relationship between mycorrhizal fungi and protocorms (Clements et al., 1979). The above pH range may also increase the synthesis of organic acids from the available carbohydrates in the medium (Dimond and Peltier, 1945).

The growth of orchids may be influenced by some environmental factors (Hugh and Thomas, 1980). Light intensity, quality and duration may affect the seed germination and seedling development of orchids (Arditti, 1967; Arditti et al., 1982; Zeiger et al., 1985). Stoutamire (1974) showed that seed germination in many plants is affected by light being either stimulatory or inhibitory depending on the wave length and the plant used.

The asymbiotic seed germination increased in the dark in case of C. elegans. Whereas, C. giganteum and T. alba exhibited higher germination in lower light intensities (Figs. 7 and 8). The seedling growth, number and area of roots and leaves in asymbiotic conditions were enhanced in the light. On the contrary, these were inhibited considerably in the dark (Tables 7-9).

In symbiotic conditions, C. elegans and T. alba exhibited better germination under dark conditions than in the light regimes but C. giganteum showed more seed germination at 1500 lux light intensity with all the fungal endophytes (Figs. 22-24). However, further symbiotic seedling growth was significantly higher in light conditions as compared to dark in all the species. Protocorms inoculated with RH36, RH54 and RA20 in C. elegans and by RH15 and RH36 endophytes in T. alba increaed the seedling growth at 1500

lux light intensity. Whereas, in C. giganteum comparatively better seedling growth was recorded with all the fungal isolates at 1500 lux light intensity (Figs. 22-24). In symbiotic condition, average number and area of roots and leaves enhanced under 1500 and 3000 lux light intensities (Tables 23 and 24).

Curtis and Nichol (1948) reported marked reduction in germination and early protocorm growth in the light. However, the growth of the late protocorms was accelerated by the light in Cymbidium and Vanda species. In Cattleya seed germination and seedling growth were promoted by light (Spoerl, 1948). Similarly Arditti et al. (1981) observed differential response of some North American orchids, to light and dark conditions for seed germination and subsequent seedling growth. Anderson (1978) reported that photosynthesis drives metabolic growth and maintenance reactions of plants, which influence the developmental process. Even at lower light regimes during the present studies, more seedling growth was observed in mycorrhizal cultures than in the controls. It may be due to their improved metabolic processes (Landecker, 1972). Light conditions may regulate, the carbohydrate synthesis, which brings about a change in their drainage through the root system. The process shall, therefore, influence the enzymatic activity of the endophyte in root region and secretion of hydrolytic exoenzymes. A reduction

in the status may bring about a change in the rate of conversion of insoluble organic substances into simpler and available forms in the root region (Smith, 1966, 1967, 1973), which could influence the development of endophyte and ultimately the seedling growth.

The maximum asymbiotic seed germination was noticed in the green and white light in C. elegans and C. giganteum, respectively. While, the seed germination was enhanced in darkness in case of T. alba. The minimum germination was obtained in the blue and red light in C. elegans, T. alba and C. giganteum, respectively (Fig. 9). The asymbiotic seedling growth index was highest under the green light in both C. elegans and T. alba. However, C. giganteum exhibited comparatively better growth index in white light than the light of other colours (Fig. 10).

Symbiotic seed germination was significantly higher using RH36 and RH54 isolates in case of C. elegans and using RH36 and RA20 in C. giganteum under red light conditions. On the other hand, T. alba showed better seed germination in the blue and red light with all the mycorrhizal fungi (Figs. 25-27). The subsequent seedling development of orchids was also increased at different colours of light in symbiotic conditions. The lowest growth index was recorded in the dark conditions in both asymbiotic and symbiotic

cultures in all the species (Fig. 10 and Tables 25-27).

Cymbidium seeds germinated well in the dark but formed small protocorms, scale-like leaves and no roots (Yates and Curtis, 1949; Kohl, 1962). When individual protocorms were transferred to complete darkness they lost their chlorophyll but produced shoots readily. These shoots were greatly etiolated (Kohl, 1962).

However, Burgeff (1932) reported that seedlings of Cymbidium species produced both shoots and roots in dark cultures with a mycorrhizal fungus. The seedlings of Oncidium seemed to require no light for both shoot and root formation. Seedlings grown in the dark appeared normal but growth was more extensive in the light (Yates and Curtis, 1949). Zeiger et al. (1985) studied the growth of Paphiopedilum species and found better growth under blue light. Similar observation was made in case of T. alba, during the present investigation. Ueda and Torikata (1972) investigated the effect of light and culture medium on adventitious root formation by Cymbidium in aseptic culture. Somewhat inferior root and leaf production was reported in red and blue light than those under green and white light in both the Cymbidium species. In the present studies, similar phenomenon was observed on root formation in Cymbidium elegans and C. giganteum in asymbiotic conditions.

The effect of light on the development of mycorrhizae in plant has been reported (Wenger, 1955; Boullard, 1961). The possible role of mycorrhizae in root development may be due to the change in carbohydrate production by the green plants. The effectiveness of different colours of light is of direct importance only when artificial light is being used for germination of seeds and growth of orchids. The best growth of orchids by using a combination of red and blue fluorescent bulbs was recorded (Withner, 1964). The combination was much better than white light alone which was next in effectiveness. Increased growth in light conditions is possible due to enhanced photosynthetic activity and a higher plant metabolic rate (Zeigler *et al.*, 1967; Lin and Molnar, 1983).

Asymbiotic seed germination, growth-index, area of seedlings/plantlets, number and area of roots and leaves of orchids were found significantly higher at photoperiod range of 8-16 hr (Figs. 11 and 12, Tables 13-15). Symbiotic seed germination was higher using RH15 and RA40 under 8 hr; RA20 under 12 hr RH36 under 20 hr and RH54 under 24 hr photoperiod in case of *C. elegans*. In *C. giganteum*, 8 and 12 hr photoperiods resulted in significantly higher seed germination (Figs. 28 and 29). *T. alba*, however, exhibited optimum seed germination under 8 hr photoperiod with all the mycorrhizal fungi. Both symbiotic and asymbiotic germination decreased at 24 hr photoperiod in all the species (Figs. 28-30). The

maximum symbiotic seedling growth index was noted under 12 hr photoperiod using different fungal symbionts in all the species while, the minimum was recorded in 24 hr photoperiod (Figs. 28-30).

Zeigler et al. (1967) investigated the influence of various media and photoperiod on growth and amino acid content of orchid seedlings and observed better growth between the photoperiod range of 12-20 hr, and suggested that enhanced growth with increasing length of photoperiods could be due to improved metabolic processes and higher photosynthetic activity resulting from longer light periods. Lin and Molnar (1983) studied the effect of photoperiod and high intensity supplementary lighting on flowering of Alstroemeria orchid and noticed higher flowering and growth upto the photoperiod range of 16 hr and then found decreasing trend.

The mycorrhizal seedlings may have better metabolic process even at low light conditions than controls (Landecker, 1972), which resulted in greater overall growth of seedlings. Hadley and Williamson (1971) have also observed an increase in growth of mycorrhizal seedlings at low level of sugars, which supported the view that mycorrhizal seedlings may comparatively grow better than non-mycorrhizal ones even in low light conditions, where the rate of carbohydrates synthesis could be low.

The production of growth substances by ectomycorrhizal fungi (Ulrich, 1960; Crafts and Miller, 1974; Ng et al., 1982), by vesicular-arbuscular mycorrhizal fungi (Barea and Aguilar, 1982) and also by orchid mycorrhizal fungi (Borroso et al., 1966) is well documented. The results of biochemical analysis of mycorrhizal fungi of orchids, indicated the presence of a maximum of five auxins, three cytokinins and six gibberellins from the seven fungal endophytes (Tables 31-33). The number and quantity of growth hormones varied in different mycorrhizal fungi at different culture periods. The amount of growth hormones increased with the age of the fungal culture, which might be due to increase in the mycelial growth (Moser, 1959). Ulrich (1960) demonstrated that amount of growth hormones, their composition and time required for their production in detectable amounts may vary between different fungal species and even between different strains (Moser, 1959). Similar results were obtained in the present investigation (Figs. 32 and 33). The improved growth of mycorrhizal plants in the present studies was expected due to increased level of growth hormones, produced by the fungal symbionts. Mycorrhizal production of growth hormones in the root zone, could also provide additional supply of these biologically active substances to the plants (Brown, 1974). The increased growth of plants may be due to the role of growth hormones in division, expansion, and differentiation of cells, RNA and protein synthesis and

many other physiological and biochemical processes (Thimann, 1969, 1972; Cleland, 1969; Skoog and Armstrong, 1970; Skoog and Schmitz, 1972).

The better growth of seedlings was observed at lower and medium doses of phosphate in the mycorrhizal sets as compared to the non-mycorrhizal controls in both the Cymbidium species (Tables 34-36). The general growth superiority of the mycorrhizal seedlings of orchids over non-mycorrhizal controls at lower phosphate levels seemed to be the direct effect of the mycorrhizae induced increase in growth of the former. Allen et al. (1981) observed significant increase in water, phosphate uptake and photosynthesis in case of Bouteloua gracilis plant in response to mycorrhizal infection, and suggested that many of these responses may be regulated in part by alterations in phytohormone levels. Abbott and Robson (1977) studied the growth stimulation of subterraneum clover with vesicular-arbuscular mycorrhizae and also noticed a marked increase in the growth and phosphorus content of mycorrhizal plants at intermediate levels of phosphate. The higher phosphorus content of the mycorrhizal seedlings compared to the controls at lower phosphate levels was due to mycorrhizal infection, which decline at the higher phosphate levels probably due to the reduction in the mycorrhizal activity (Thomas et al., 1982). The increased phosphorus concentration in the mycorrhizal

seedlings might be due to increased absorption area contributed by the fungal hyphae (Pearson et al., 1975) and increased phosphatase activity in the roots (Allen et al., 1981).

Initially, the phosphatase activity was significantly higher and then decreased with the increase of seedlings-age in both the Cymbidium species (Figs. 36 and 37). Gianinazzi et al. (1978) studied phosphatase activities in the roots of onion seedlings and noticed more or less similar results. The acid and alkaline phosphatase activities decreased in both the mycorrhizal and non-mycorrhizal seedlings of orchids with the increase of phosphate levels in the soil. However, mycorrhizal seedlings exhibited more acid phosphtase than the non-mycorrhizal ones in the control sets (Figs. 36 and 37). Williamson et al. (1975) studied acid phosphatase activity in the fungal sheath of beech mycorrhizae and observed upto eight times more phosphatase activity on the surface of mycorrhizae and suggested that this activity was important in the uptake and translocation of phosphorus by mycorrhizae (Bartlett and Lewis, 1973). Gianinazzi and Gianinazzi (1976) observed slightly higher acid phosphatase activity in the phosphorus-deficient non-mycorrhizal roots than those supplied with soluble phosphorus. A much greater stimulation of acid phosphatase activity in roots of plants grown under phosphorus deficient conditions has also been reported by

Woolhouse (1969). Bieleski and Johnson (1972) investigated phosphatase activity in phosphorus-deficient Spirodela oligorrhiza and demonstrated that inorganic phosphate deficiency may promote phosphatase activity. The phosphorus deficient plants may have more demand for phosphorus due to increased phosphatase activities. Williamson (1973) studied acid phosphatase and esterase activities in orchid mycorrhizae and suggested that large increase in phosphatase activity resulted from the depletion of phosphate-ester pools or other nutrients in cells. Gianinazzi et al. (1979) investigated localization of phosphatase enzyme in vesicular-arbuscular mycorrhiza in the onion roots and noticed no marked modifications in the acid phosphatase activity either of the host cells or within the arbuscule branches after fungal infection. As the fungus become more mature, acid phosphatase activity became less and less evident. But as the hyphae matured, alkaline phosphatase activity increases and was more intense. The alkaline phosphatase activity, therefore, seemed to be involved, in the active phosphate transport mechanisms (Cox et al., 1975; Tinker, 1975).

SUMMARY

Effects of different ecophysiological factors, i.e., temperature, pH of the medium and light on asymbiotic and symbiotic seed germination and subsequent seedling development of three orchid species, viz., Cymbidium elegans (Blume), C. giganteum (Wall.) and Thunia alba (Rchb.f.) were studied. Different parameters like, percentage of germination, growth index, area of seedlings/plantlets, number and area of leaf primordia/leaves and number and area of rhizoids/roots were used. The effect of different temperature (20, 25, 30 and 35°C) on seed germination and seedling growth in asymbiotic conditions was studied. Maximum germination of seeds was observed at 20°C in case of C. elegans, whereas, C. giganteum and T. alba showed highest germination at 25°C. The higher temperatures were found inhibitory. Vigorous growth of seedlings occurred between temperature range of 20-30°C in all the cases, but it was markedly reduced at 35°C. Statistically significant variations were obtained in seed germination and seedling growth at different temperatures in all the species. Asymbiotic seed germination and seedling growth were studied at different pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0) levels of modified Kn C medium. The higher germination and better seedling growth were recorded at pH 4.0 and 5.0, whereas, the pH 10.0 proved detrimental for germination and protocorm growth. The maximum number and area of leaf primordia/leaves and also rhizoids/roots were noticed at pH 4.0, 5.0 and 6.0 in C. elegans, C. giganteum and T. alba,

respectively. Significant variations were observed in seed germination and seedling growth at different pH levels.

The effect of different light intensities (1500 lux, 3000 lux, 5000 lux, and 0 lux), light qualities (red, green, blue, white and dark) and photoperiods (8 hr, 12 hr, 16 hr, 20 hr and 24 hr) on aseptic seed germination and growth of seedlings of orchids were investigated. Seed germination was comparatively better in the dark conditions in C. elegans and T. alba, while in C. giganteum, the higher percentage of seed germination was recorded in the light conditions. However, subsequent seedling growth significantly increased under the light conditions in all the species studied. The highest seedling growth index was observed under 1500 lux in C. elegans and under 3000 lux light intensity in C. giganteum and T. alba, while, the lowest growth index was observed in the dark in all the species. The formation and development of roots and leaves asymbiotically were also enhanced at 1500 and 3000 lux light intensities in all the cases.

The green and white light regimes exhibited comparatively higher seedling growth index, area of seedlings/plantlets, number and area of rhizoids/roots and number and area of leaf primordia/leaves in C. elegans, T. alba and C. giganteum, respectively. The asymbiotic seed germination was better at photoperiods of 8-16 hr but it considerably decreased at 20 and 24 hr photoperiods in all the orchid

species. The subsequent seedling growth was significantly higher at 12 hr photoperiod in C. elegans and T. alba while in C. giganteum better seedling development occurred in 16 hr photoperiod. Statistically significant variations were observed in seed germination and seedling growth between different light treatments.

Five-orchid-mycorrhizal fungi (RA20, RA40, RH15, RH36 and RH54) were selected for in vitro symbiotic studies in C. elegans, C. giganteum and T. alba on the oat medium. The inoculated cultures exhibited significantly higher seed germination and subsequent seedling growth than the uninoculated controls at various temperatures, pH levels of the medium and light conditions. All the fungal isolates enhanced the germination and growth of seedlings/plantlets between the temperature range of 20-30°C than the other temperatures. The symbiotic seed germination and seedling development of orchids were greatly reduced at 35°C. Significant variations were observed between fungal isolates and different temperatures. Seed germination in C. elegans was higher at pH 3.0 using RH36, RA40 and at pH 4.0 using RH15, RH54 and RA20 strains. In C. giganteum at 4.0 using RH36, RH54 and at pH 5.0 with RH15, RA20 and RA40 isolates higher germination was recorded. T. alba, however, exhibited the maximum germination at 6.0 pH with all the fungal symbionts. The minimum symbiotic seed germination was recorded at pH 10.0 in all the species. The

symbiotic growth index, area of seedlings/plantlets and number and area of leaf primordia/leaves and rhizoids/roots were enhanced in the pH range of 4.0 - 6.0 but these decreased at pH 8.0 and 10.0 in all the cases. The variations between the fungal isolates and different pH levels were found to be significant.

The effect of three light intensities, i.e., 1500 lux, 3000 lux, 5000 lux and dark was studied on seed germination and seedling growth of orchids in symbiotic condition. The moderate light intensity (1500-3000 lux) enhanced the growth of mycorrhizal seedlings than controls in all the cases. The seedlings inoculated with mycorrhizal fungi exhibited better growth even at low light intensity in comparison to control provided with even higher light intensities.

The symbiotic growth of seedlings was comparatively higher under white and green light in C. elegans and under red and green light in C. giganteum and T. alba than the light of other colours. Likewise, the formation and development of roots and leaves also enhanced in the green and white light conditions, with all the fungal endophytes in all the species studied. The seedling development of orchids was retarded in the dark.

The maximum germination of seeds occurred in photo-

period range of 8-16 hr with all the fungal symbionts. However, symbiotic seed germination was considerably lower at 24 hr photoperiod. The average area of seedlings/plantlets and number and area of leaf primordia/leaves and rhizoids/roots in symbiotic condition were higher under 12 and 16 hr photoperiod in C. elegans, T. alba and C. giganteum, respectively. Significant variations were observed between different light conditions.

The results of biochemical analysis of mycorrhizal fungi of orchids indicated the presence of maximum five auxins, three cytokinins and six gibberellins. The number and quantity of these growth substances increased with the growth of the mycorrhizal fungi in pure cultures. The quantitative study of fungal extract exhibited that the maximum amount of auxins was noticed in RH51 followed by RH61 and least in RH36 isolate. Similarly, the amount of gibberellins was also significantly higher in RH51 and lowest in RH36 strains. The RH15 and RH46 endophytes produced the highest and lowest quantity of cytokinins, respectively in pure culture at the age of sixty days.

The growth of mycorrhizal and control (non-mycorrhizal) seedlings of Cymbidium elegans and C. giganteum in the earthen pots was studied at different phosphate levels of the soil mixture. The overall growth of seedlings in both

the species was significantly higher in the inoculated sets than in the controls at lower and medium doses of phosphate. The non-mycorrhizal seedlings, however, showed the increasing trend with the increase of phosphate levels in the soil mixture in both the cases.

The endogenous phosphorus content of mycorrhizal seedlings of C. elegans significantly increased upto 3.36 mg/pot phosphate level and then showed a decreasing trend. But the phosphorus content of the controls increased with the increase in the phosphate levels. Whereas, inoculated seedlings of C. giganteum exhibited increasing trend of phosphorus content upto 33.6 mg/pot phosphate level it declined at higher levels. The control seedlings of this species showed better phosphorus content at the highest phosphate level (67.2 mg/pot).

At 30 days of seedling growth, the phosphatase activity was significantly higher and it subsequently decreased with the increase of seedlings-age in both the Cymbidium species. In cases where phosphate was not supplied, the mycorrhizal seedlings exhibited higher acid phosphatase activity than in the non-mycorrhizal ones. Whereas, the uninoculated seedlings showed more alkaline phosphatase activity than the inoculated ones in cases where phosphate was not added to the soil. The acid and alkaline phosphatase

activities decreased in both the mycorrhizal and non-mycorrhizal seedlings with the increase of phosphate levels in the soil.

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List of Publications of Shri A.N. Raghuwanshi

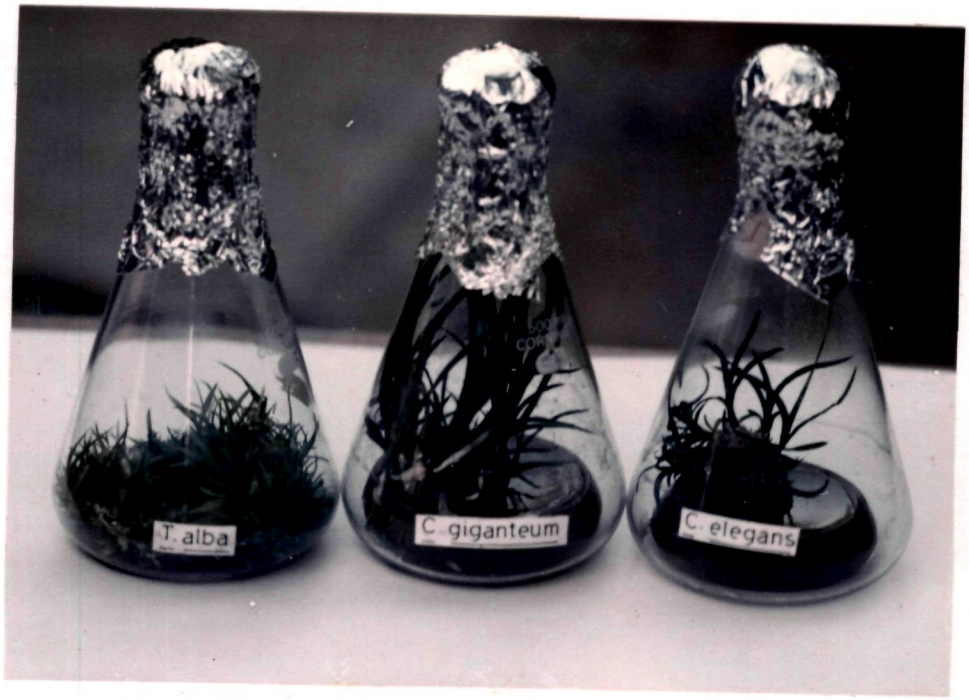
1. Raghuwanshi, A.N., Mishra, R.R., Tandon, P. and Sharma, G.D. (1983). Studies on asymbiotic germination of orchid seeds. J. Meghalaya Sci. Soc. 7 and 8: 1-5.

2. Raghuwanshi, A.N., Mishra, R.R. and Sharma, G.D. (1985). Effect of synthetic media on asymbiotic seed germination and seedling growth of *Dendrobium nobile* and *Sarcanthus pallidus*. Proc. Ind. Nat. Sci. Acad. B51: 360-363.

3. Raghuwanshi, A.N., Mishra, R.R. and Sharma, G.D. (1986). Effect of pH on asymbiotic seed germination and seedling development of orchids. In: Biology, Conservation and Culture of Orchids (ed. S.P. Vij), Affiliated East-West Press Private Limited. pp.453-462.

4. Raghuwanshi, A.N., Mishra, R.R. and Sharma, G.D. Effect of temperature on asymbiotic seed germination and seedling growth of orchids. Indian J. Ecol. (In Press).

5. Raghuwanshi, A.N., Mishra, R.R. and Sharma, G.D. Effect of pH of the medium on asymbiotic seed germination and seedling growth of *Dendrobium moschatum*. The J. Environ. Sci. (In press).


T. alba
C. giganteum
C. elegans