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**G Arsha**
Assistant Professor (Plant Pathology), Department of Crop Protection, Palar Agricultural College, Kothamarikuppam, Tamil Nadu, India

**CS Karibasappa**
Assistant Professor (Plant Pathology), Department of Crop Protection, Palar Agricultural College, Kothamarikuppam, Tamil Nadu, India

**LDC Henry**
Associate Professor, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

**K Sridhar**
Associate Professor (Plant Pathology), Dept. of Crop Protection, Palar Agricultural College, Kothamarikuppam, Tamil Nadu, India

**Corresponding Author:**
**G Arsha**
Assistant Professor (Plant Pathology), Department of Crop Protection, Palar Agricultural College, Kothamarikuppam, Tamil Nadu, India

# A comprehensive study of culture origin, media, pH, and nitrogen supplements on vegetative growth of *Volvariella volvacea*

**G Arsha, CS Karibasappa, LDC Henry and K Sridhar**

**Abstract**
Mushrooms are abundant in proteins, vitamins, and minerals, which has led to them being commonly referred to as the "meat of the vegetarians." This research aimed to determine the ideal pH, appropriate growth medium, and nitrogen source for promoting the in vitro vegetative growth of *Volvariella volvacea.* The cultures developed from the tissue taken from the junction of pileus and stipe excelled by recording the maximum yield and biological efficiency. *V. volvacea* was found to grow best in PDA medium followed by carrot dextrose agar medium at a pH of 6 at the optimum temperature of 34 °C. In addition, peptone as nitrogen source supported the maximum mycelial growth of *Volvariella volvacea.*

**Keywords:** *Volvariella volvacea*, pileus, stipe, mushroom

## Introduction
Mushrooms are defined as “macro fungi with a distinctive fruiting body, large enough to be seen with the naked eye and to be picked up by hand” (Chang and Miles, 1991). Mushrooms contribute a substantial nutritional boost to the diet, supplying proteins, carbohydrates, essential minerals, vitamins, and valuable salts, all while being low in fat and calories. To sustain a proper nutritional equilibrium, an average individual should consume between 100 to 200 grams of mushrooms per day. Mushrooms also have high medicinal value and their pharmacological action and therapeutic properties in promoting human health are known for thousands of years. Furthermore, the cultivation of edible mushrooms has gained recognition as a straightforward on-farm technique for the profitable transformation and reuse of diverse lignocellulosic agricultural, industrial, and forestry residues.
Paddy straw mushroom (*Volvariella volvacea*) is a popular variety among people because of its distinct flavor, pleasant tastes, higher protein content and shorter cropping duration compared to other cultivated mushrooms. It has been reported that the present production of paddy straw mushroom in India is almost negligible and only small quantities are produced in Orissa, Tamil Nadu and Kerala. Hence, the present investigations were conducted with an objective to study the different cultural practices and their effect on the enhancement on the vegetative growth and yield of *V. volvacea.*

## Material and Methods
This investigation was carried out at the Department of Plant Pathology, Annamalai University and Research laboratory of Biotehnology in Care Keralam, Kerala during 2020-2022.

### Performance of *V. volvacea* cultures obtained from various regions of the sporocarp
The tissues obtained from the stipe, pileus, junction of pileus and stipe and volva of the mushroom were maintained in PDA medium. Paddy grain spawn was prepared using the pure cultures of *V. volvacea* following the method described by Sivaprakasam (1980) [7]. For each treatment three replications were maintained. Mushrooms were cultivated using hollow interior bed system. The observations on the yield attributes like spawn run days, days taken for pin head formation, average weight, number of sporocarp, yield and biological efficiency were assessed and recorded. The biological efficiency of *V. volvacea* was calculated by the following formula

$$BE = \frac{\text{Fresh weight of the mushroom / bed}}{\text{Dry weight of the substrate / bed}} \times 100$$

**Effect of different culture media on the vegetative growth of *V. volvacea***

Six distinct media namely potato dextrose agar (PDA), carrot dextrose agar (CDA), beetroot dextrose agar (BRDA), bean dextrose agar (BDA), yam dextrose agar (YDA) and rice dextrose agar (RDA) were assessed for their effectiveness in promoting the expansion of *V. volvacea* mycelium. Each medium was taken separately in Petri plates with a volume of 15 ml and allowed to cool. Subsequently, the plates were each inoculated with mycelial discs measuring nine millimeters in diameter derived from a vigorously growing seven-day-old *V. volvacea* culture. These inoculated plates were then placed at room temperature (28±2 °C) for incubation. This experimental process was replicated three times. The radial growth of the mycelium was regularly measured over an eight-day period periodically and expressed in millimeters.

To assess the fungal biomass 50 ml of different broth were prepared and taken in conical flasks. They were inoculated with nine mm mycelial discs aseptically. Upon the completion of the incubation period (spanning 15 days) the mycelial mat was filtered using pre-weighed Whatman No. 1 filter paper. Subsequently, the filtered mycelial mat was subjected to drying within a hot air oven set at 105 °C until a consistent weight was achieved, and this weight was duly recorded. The density of chlamydospores present was evaluated and categorized into levels: low, medium, high and very high. The determined chlamydospore density level was then documented for further reference.

**Effect of pH on the vegetative growth of *V. volvacea***

The pH levels of both potato dextrose agar (PDA) and potato agar broth were adjusted to values of 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. These different pH conditions were then examined for their effectiveness in facilitating the growth of *V. volvacea* mycelium. The progress of fungal growth was regularly monitored on the 3rd, 5th, and 8th days. The observation of fungal biomass was concluded on the 15th day after inoculation and was quantified in terms of millimeters and grams. The most suitable pH 6 was used for further experiments.

**Effect of varied nitrogen suppliments on the *in vitro* growth of *V. volvacea***

Different nitrogen supplements including ammonium sulfate, urea, peptone, DAP and yeast extract were introduced into PDA medium at a concentration of 2% each. This was carried out to assess their impact on the vegetative growth of *V. volvacea.* To achieve a 2% concentration of these additives, two grams of each were individually added to 100 ml of PDA medium. A control medium without any supplements was used as a reference and each treatment was replicated three times.

**Quantification of Mycelial Growth**

The extent of radial expansion of *V. volvacea* mycelium across different media was determined through the measurement of mycelium diameter within the Petri dish.

**Results and Discussions**

**Performance of *V. volvacea* cultures obtained from various regions of the sporocarp**

The data regarding performance of tissue culture isolates obtained from various regions of the sporocarp on the yield parameters and biological efficiency are presented in table 1. Among the different cultures tested, the cultures developed from the tissue taken from the junction of pileus and stipe excelled the cultures from other regions by recording the minimum spawn run days (10.1 days), minimum days for the pin head formation (4.0), the maximum number of sporophores (14.1), the maximum yield (307.0 g/bed) and the maximum biological efficiency (30.7%) followed by the cultures obtained from the spores, pileus, stipe and volva region in the decreasing order of merit. The culture obtained from volva region recorded the maximum number of spawn run period (15.3 days) and the minimum sporophore yield of 264.0 g per bed when compared to other cultures tested.

The better performance of the cultures obtained from the junction of pileus and stipe region observed in the present study could be attributed to the presence of more active, viable and virulent cells in that region as reported by Kapoor (1989) [4] who stated that more of actively differentiating cells could be seen at the junction of pileus and stipe of a sporophore and attributed the same for the better performance of the cultures.

**Table 1:** Performance of *V. volvacea* cultures obtained from various regions of the sporocarp

| Tr. No | Source | DFSR | DFPF | Wt. of sporophore (g) | No of Sporophore/bed | Yield (g/bed) | B.E (%) |
|---|---|---|---|---|---|---|---|
| 1 | Spores | 10.3±0.16$^{a}$ | 4±0.65$^{a}$ | 16.2±0.80$^{a}$ | 13.4±0.40$^{b}$ | 299±48$^{ab}$ | 29.9$^{ab}$ |
| 2 | Pileus | 11.0±0.08$^{b}$ | 5±0.35$^{b}$ | 14.8±0.40$^{b}$ | 13.2±0.20$^{b}$ | 295±2.4$^{ab}$ | 29.5$^{ab}$ |
| 3 | Stipe | 12.3±0.04$^{c}$ | 5±0.15$^{b}$ | 14.3±0.20$^{b}$ | 13.1±0.10$^{b}$ | 285±1.2$^{bc}$ | 28.5$^{bc}$ |
| 4 | Volva | 15.3±0.03$^{d}$ | 6±0.15$^{c}$ | 11.9±0.14$^{c}$ | 12.6±0.06$^{c}$ | 264±0.8$^{c}$ | 26.4$^{c}$ |
| 5 | Junction of pileus & stipe | 10.1±0.18$^{a}$ | 4±0.65$^{a}$ | 15.8±0.80$^{a}$ | 14.1±0.40$^{a}$ | 307±4.0$^{a}$ | 30.7$^{a}$ |

DFSR: Days for spawn run

DFPF: Days for pin head formation

*mean of three replication

*values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)

**Effect of culture media on the vegetative growth of *V. volvacea***

Out of the various solid and liquid media examined Potato Dextrose Agar (PDA) medium exhibited the highest radial growth, biomass production and chlamydospore formation recording (90.0 mm & 1.02 g) followed by carrot agar medium (86.0 mm & 0.89 g) respectively on the eighth day of observation. The radial growth of the mycelia in media *viz.*, beetroot agar media (85.89 mm), Rice agar media (85.2 mm) and yam agar media (84.1 mm) were on par with each other. Bean agar medium was found to be inferior with regard to the biomass production (0.76 g), minimum radial growth (80.3 mm) and the least density of chlamydospores (Table 2 and Plate 1).

The outcomes align with the observations of Kumar *et al.* (2016) [15], who documented the highest mycelial growth of *V. volvacea* in potato agar media.

**Table 2:** Effect of culture media on the vegetative growth of *V. volvacea*

| Tr. No | Medium | Radial growth (mm) | | | Mycelial dry weight (g) | Chlamydospore density |
|---|---|---|---|---|---|---|
| | | 3rd day | 5th day | 8th day | | |
| 1 | Potato Dextrose Agar | 33.3±0.44$^{a}$ | 71.5±0.54$^{a}$ | 90.0±0.89$^{a}$ | 1.02±0.03$^{a}$ | +++ |
| 2 | Carrot Dextrose Agar | 30.5±0.22$^{b}$ | 68.3±0.33$^{a}$ | 86.0±0.56$^{b}$ | 0.89±0.02$^{b}$ | +++ |
| 3 | Beetroot Dextrose Agar | 30.2±0.12$^{bc}$ | 67.9±0.12$^{a}$ | 85.89±0.23$^{b}$ | 0.85±0.04b$^{c}$ | +++ |
| 4 | Bean Dextrose Agar | 24.6±0.89$^{d}$ | 56.3±1.07$^{b}$ | 80.3±1.78$^{d}$ | 0.76±0.05$^{d}$ | + |
| 5 | Yam Dextrose Agar | 28.4±0.44$^{c}$ | 58.7±0.54$^{b}$ | 84.1±0.89$^{c}$ | 0.80±0.03$^{bc}$ | ++ |
| 6 | Rice Dextrose Agar | 29.5±0.89$^{bc}$ | 68.1±1.07$^{a}$ | 85.2±1.78$^{c}$ | 0.83±0.04$^{cd}$ | +++ |

+ = Low, ++ = Medium, +++ = High, +++ = Very high

* mean of three replication

* Values without a shared superscript differ significantly at $p<0.05$ according to DMRT.

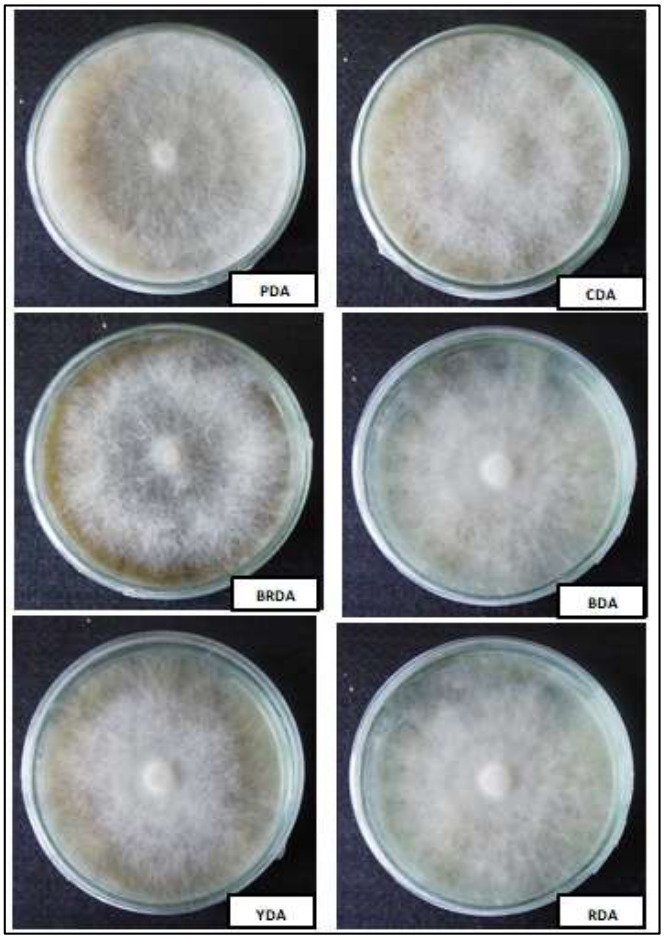


**Plate 1:** Effect of culture media on the vegetative growth of *V. volvacea*

**Effect of pH on the vegetative growth of *V. volvacea***

The data presented in table 3 highlights that the maximum biomass production of *V. volvacea* (1.23 g) was obtained at pH 6.0 after 15$^{th}$ day of incubation followed by pH 6.5 (1.20 g) and pH 5.5 (1.15 g). Similarly, pH 6.0, 6.5 and 7.0 recorded a mycelial extension of 90.0 mm on the 8$^{th}$ day after inoculation in the solid medium. It is noteworthy that an increase in pH above 7.5 and below 5.5 showed significant reduction in the mycelial growth and biomass production of *V. volvacea*. (Fig 1 and Plate 2).

The growth of any organism is significantly influenced by the pH of the medium. It has been firmly established that the concentration of hydrogen ions (pH) in the growth media impacts the growth and metabolism of mushroom fungi. For maximum biomass production by *V. volvacea*, an optimal pH of 6.5 has been determined (Bellettini *et al.*, 2019) [1]. Hopkins (1995) [3] noted that extremely acidic or alkaline pH levels could lead to cell wall corrosion and disruption of the selective permeability function of the cell membrane. This could explain the substantial decline in mycelial growth when pH falls below 5.5 or rises above 7.5. The current findings are consistent with these earlier reports.

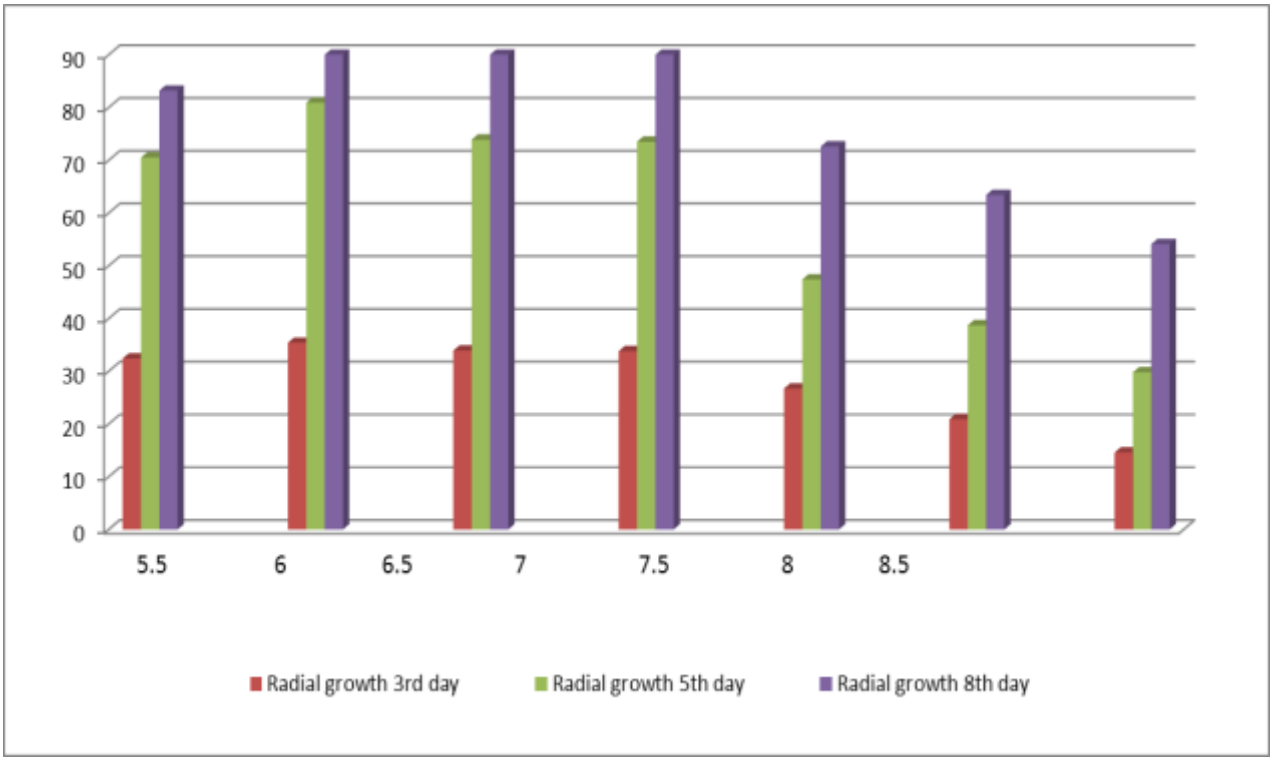


**Fig 1:** Effect of pH on the vegetative growth of *V. volvacea*

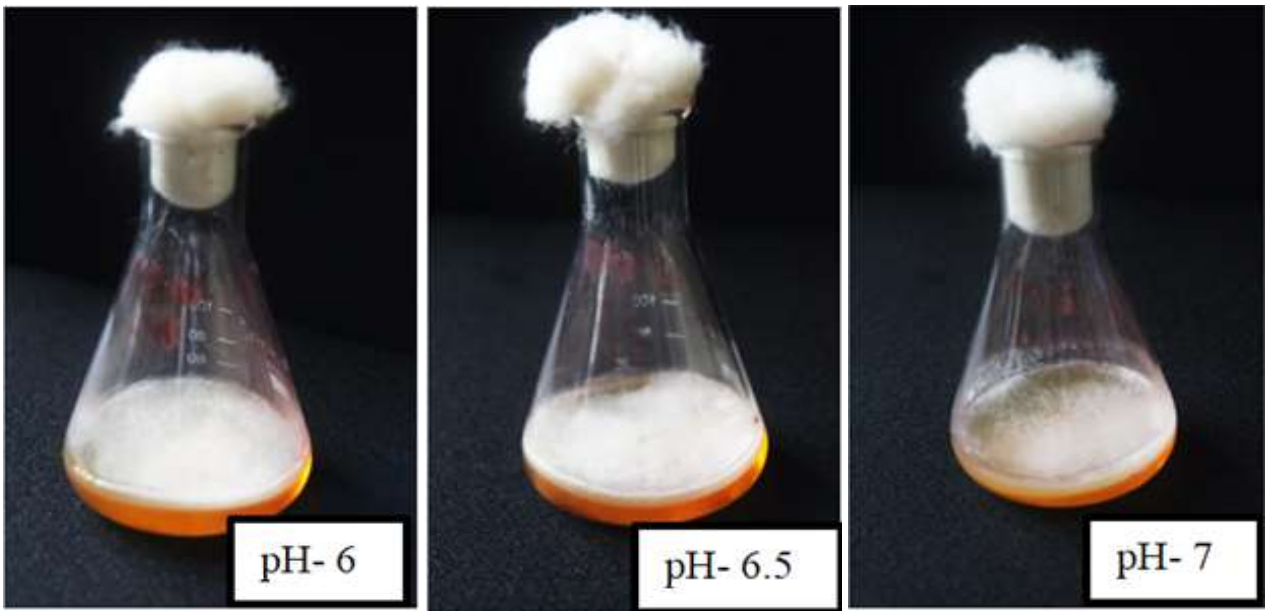


**Plate 2:** Effect of pH on the vegetative growth of *V. volvacea*

**Effect of varied nitrogen supplements on the *in vitro* growth of *V. volvacea***

The data presented in Table 3 clearly indicates that media enriched with different nitrogen sources, namely ammonium sulfate, urea, peptone, DAP and yeast extract, exhibited enhanced fungal mycelial growth compared to the control. The fungus displayed its most rapid growth and highest chlamydospore density in the medium supplemented with peptone (5.9 days and 1.32 g) followed by yeast extract (6.1 days and 1.26 g) ammonium sulfate (6.2 days and 1.25 g) and DAP (6.3 days and 1.33 g). Conversely, the inclusion of urea in the PDA medium led to limited mycelial growth and chlamydospore formation.

According to Rangaswamy (1956) [6], the inclusion of peptone in the media amplified both the vegetative growth and chlamydospore density of *V. volvacea*. The introduction of nitrogen can elevate oyster mushroom yield; however an excessive amount can lead to reduced yield as an excess of nitrogen hampers the mushroom's fruiting process (Bellettini *et al.*, 2019) [1].

**Table 3:** Effect of varied nitrogen supplements on the in vitro growth of *V. volvacea*

| Tr. No. | Additives (@ 2% level) | Days taken to cover 90 mm Dia Petri plate | Mycelial dry weight (g) | Chlamydospore formation (days) | Chlamydospore density |
|---|---|---|---|---|---|
| 1 | Ammonium sulphate | 6.2±0.15$^{b}$ | 1.25±0.111$^{b}$ | 11.8±0.15$^{b}$ | ++++ |
| 2 | Urea | 6.7±0.06$^{c}$ | 1.24±0.06$^{c}$ | 12.4±0.04$^{c}$ | ++ |
| 3 | Peptone | 5.9±0.06$^{a}$ | 1.32±0.1$^{a}$ | 11.5±0.03$^{b}$ | ++++ |
| 4 | DAP | 6.3±0.07$^{bc}$ | 1.33±0.04$^{a}$ | 13.2±0.02$^{d}$ | +++ |
| 5 | Yeast extract | 6.1±0.02$^{b}$ | 1.26±0.1$^{b}$ | 11.6±0.02$^{b}$ | +++ |
| 6 | Control | 7.8±0.08 $^{d}$ | 1.23±0.06$^{c}$ | 13.8±0.02$^{d}$ | ++++ |

* Mean of three replication
* Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)
* Values without a shared superscript differ significantly at $p<0.05$ according to DMRT

## Conclusion

*V. volvacea*, characterized by its rapid growth, thrives optimally in solid-phase potato dextrose agar medium with a pH of 6.0. The nitrogen source peptone exhibited the highest support for the mycelial growth of *Volvariella volvacea.* The cultures developed from the tissue taken from the junction of pileus and stipe excelled by recording the maximum yield and biological efficiency

## Acknowledgement

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## References

1. Bellettini MB, Fiorda FA, Maieves HA, Teixeira GL, Ávila S, Hornung PS, *et al*. Factors affecting mushroom *Pleurotus* spp. Saudi Journal of Biological Sciences. 2019;26(4):633-646.
2. Chang ST, Miles PG. Recent trends in world production of cultivated edible mushrooms. Mushroom. 1991;504:15-18.
3. Hopkins WG. Introduction to plant physiology. John Wiley, sons Inc. USA; c1995. p. 73-74.
4. Kapoor JN. Mushroom cultivation. Publications and Information Division. ICAR, New Delhi; c1989. p. 1-84.
5. Kumar NK, Krishnamoorthy AS, Akannon AK, Amirthan D. Influence of temperature and pH on mycelial growth and chlamydospore production of paddy straw mushroom (*Volvariella volvacea*) (Bull.Ex.Fr.) Sing. The Journal of Research ANGRAU 2016;44(1&2):1-7.
6. Rangaswami G. Studies on *Volvariella diplasia* (Berk& Br.) Madras Agricultural Journal. 1956;43:182-191.
7. Sivaprakasam K. Studies on oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer. Ph.D. Thesis, Tamil Nadu Agricultural University, India; c1980.