MATERIAL AND METHODS

This study includes investigations on many facets and the procedures followed in various experiments are presented under two subheads:

Microbiological methods

The genus *Morchella* as reviewed earlier includes many species distributed in different parts of the world, taxonomic status of some of them being in doubt. In the present investigations locally available species were used excepting *Morchella crassipes* which was obtained from United States of America. *Verpa bohemica* a species closely associated with the genus in the valley was also included in different studies. Pure cultures of the various species were obtained in the following way:

Inside-tissue from the hollow stipe of a freshly collected fruit body was obtained and given surface disinfection by either dipping it in 70% alcohol or in mercuric chloride (1:1000). Those disinfected with alcohol were flamed to remove the alcohol and planted directly on malt agar tubes. Mercuric chloride disinfected ones were given the usual washes in sterile distilled water and then planted on malt agar tubes. The timing of the treatments were carefully adjusted to avoid any damage.
to the tissue. Mycelium was transferred to fresh tubes as soon as it appeared. Cultures were incubated for sometime at 25°C and then stored in a refrigerator at 2-5°C. Stock cultures were maintained on potato-dextrose agar on which all the cultures gave good growth and were subcultured every month. A list of the species along with their source used in cultural, physiological and nutritional studies in the present investigations is given in Table I.

Table I  **LIST OF THE TESTED MOREL SPECIES**

<table>
<thead>
<tr>
<th>Morchella species</th>
<th>Date &amp; year of isolation</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morchella conica</td>
<td>12.4.1966</td>
<td>Conifer forest floor, Hing forests, Shaderwah (Jammu).</td>
</tr>
<tr>
<td>Morchella deliciosa I</td>
<td>29.4.1966</td>
<td>Pine forest floor, Pahalgam (Kashmir).</td>
</tr>
<tr>
<td>Morchella deliciosa II</td>
<td>18.4.1970</td>
<td>Pine forest floor, Sedouh, Shopian (Kashmir).</td>
</tr>
<tr>
<td>Morchella hybrida</td>
<td>30.4.1966</td>
<td>Fir forest floor, Hayan, Kangan (Kashmir).</td>
</tr>
<tr>
<td>Morchella esculenta I</td>
<td>12.8.1964</td>
<td>Conifer forest floor, Haputnar, Romsh range (Kashmir).</td>
</tr>
<tr>
<td>Morchella esculenta II</td>
<td>14.4.1966</td>
<td>Pine forest floor, Patnitop, Batote (Jammu).</td>
</tr>
<tr>
<td>Morchella angusticeps</td>
<td>5.4.1970</td>
<td>Side of a rivulet, Bursulla, Srinagar (Kashmir)</td>
</tr>
<tr>
<td>Morchella crassipes</td>
<td>2.4.1964</td>
<td>Obtained from U.S.D.A. Northern utilization Research Development Peoria, Illinois, U.S.A.</td>
</tr>
<tr>
<td>Verpa bohemica</td>
<td>19.4.1970</td>
<td>Fir forest floor, Hayan, Kangan (Kashmir).</td>
</tr>
</tbody>
</table>
Nomenclature and determinations of the different species was according to Seaver (1942).

In physiological experiments Pyrex or Corning glassware was used. Prior to use all glass vessels were cleaned with sulphuric acid dichromate solution for maximally half an hour, then thoroughly rinsed with hot water and allowed to stand filled with hot water for about 24 hours. Subsequently they were rinsed with distilled water. 25 ml of nutrient media was used per 150 ml Erlenmeyer flask in the studies unless stated otherwise. The flasks were stoppered with nonabsorbent cotton plugs protected by a piece of muslin cloth to prevent fall of loose cotton fibres in media. Solid media were autoclaved at 15 lb pressure for 20 minutes and the liquid media for nutritional studies at 15 lb pressure for 5 minutes, to avoid brown-colouring of the solutions. Petri plates were sterilized for one hour in a hot air oven at 160°C.

The cultures were incubated at 26 ± 2°C, except in temperature studies. Each treatment was replicated thrice unless otherwise stated. Dry weights were determined after ten days of growth. Mycelium was harvested under vacuum on a Buchner funnel through Whatman No. 40 filter paper discs (12.5 cm) which had been previously dried in a hot air oven at 80°C and weighed. The mycelia adhering to the bottom and sides of the flask were scraped free with a rubber policeman. Mycelium was washed three times with portions of hot distilled water to remove any residual sugar,
minerals or other materials which might give an erroneous mycelial weight. Where starch was used in the medium, the mycelia were floated out in large volumes of water before filtering, to remove as much as possible of the unused starch. The filter papers along with the mycelial mats were dried at 90°C in an oven for 24-48 hours, cooled in a desiccator over anhydrous calcium chloride for one hour and weighed immediately on an analytical balance. After the first weighing the filter papers were dried for an additional 24 hours and weighed again. This procedure was repeated until weights constant to within 2-5 mg were obtained. The cultures were incubated in a stationary position because of the variable effects of shaking. Average values of the replicates in treatments were worked out and used as quantitative measure for comparing the growth under different treatments. Cultures were exposed to diffuse daylight with intervening dark periods at night. In case of temperature studies, where the flasks and tubes were put in incubators, the cultures were exposed to laboratory light for approximately one hour 3, 5 & 7 days after inoculation (Robbins & Hervey, 1965).

Inoculations were made directly from 2.5% malt agar plates. Mycelial discs 6 mm in diameter were cut from periphery of 4 day old mycelial cultures with sterile cork borer and one of these discs was used to inoculate each flask. pH was determined by Phillips pH meter. Analytical grade reagents were used.
The basal medium for nutritional studies was medium A—a modification of that used by Fries (1955) in her physiological studies on the genus Cordyceps. Organic carbon compounds were prepared so that they would yield the grams of carbon equivalent to that in 10 g of glucose per litre of medium. Nitrogen compounds were prepared so that they would yield 250 milligrams of nitrogen per 1000 ml nutrient solution. Polysaccharides were used in the concentration of 10 g per litre. The method of separate sterilization in carbon and nitrogen sources was utilized to avoid any possible chemical interaction or breakdown of the sugars and amino acids with resultant inhibitory or stimulatory effects (vide Lopes & Fergus, 1966). Heat sensitive sugars and organic nitrogen sources were sterilized by subjecting them to steaming for 30 minutes per day for three consecutive days. Urea was sterilized by filtration through Gs filter to avoid decomposition. No attempt was made in carbon-nitrogen utilization experiments to maintain pH within narrow limits by buffer system because such system creates a highly artificial environment (Sorensen & Hesselstine, 1966). The combined solution wherever necessary was aseptically adjusted to pH 6.8-7.0 by adding sterile 0.1N HCl or 0.1N NaOH after determining the acid or base required. The pH of the culture medium was determined at the time of harvest on the joined liquids from all flasks in the same series.

The influence of temperature on fungal growth was studied by incubating the cultures in incubators adjusted to different temperature levels. In order to avoid the
lag effect the flasks were incubated at the particular temperature at least 24 hours before inoculation. For high and low temperatures observations were recorded on Potato-dextrose agar tubes.

Germination studies at different pH values were carried out by sowing spores in phosphate buffers on sterile microscope slides placed in petri dishes in a saturated atmosphere, pH of the habitat samples were determined by the method described for other soil samples subsequently.

The cultural studies of morel species on various agar media were carried out by plating equal quantities of medium in 10 cm petri dishes and inoculating with 6 mm mycelial discs.

Statistical analysis:

The dry weights of the fungal mats were statistically analysed. The standard error was calculated by the formula:

\[
\text{Standard Error (S.E.)} = \sqrt{\frac{\text{Mean square of the error}}{\text{Number of replicates}}}
\]

and the critical difference (C.D.) was determined by applying the formula:

\[
\text{Critical Difference (C.D.)} = S.E. \times t \times \sqrt{2}
\]

Media used and their constitution:

<table>
<thead>
<tr>
<th>Media Name</th>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato-Dextrose Agar</td>
<td>Peeled and sliced potatoes</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Dextrose</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Agar</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Distilled water to make</td>
<td>... 1000 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 g</td>
</tr>
</tbody>
</table>
Potatoes were cooked for one hour in 500 ml distilled water and potato extract obtained by decanting. 20 g agar was dissolved in 500 ml distilled water. Two solutions were mixed to which 20 g of dextrose were added and then autoclaved.

**Malt Agar**

- **Malt extract**
  - 25 g
- **Agar**
  - 20 g
- **Distilled water to make**
  - 1000 ml

Dissolved the malt extract in 500 ml of hot distilled water. Melted the agar in 500 ml distilled water. Mixed the two and autoclaved.

**Brock’s Agar (Brock, 1951)**

- **Glucose**
  - 30 g
- **Sodium nitrate**
  - 1.5 g
- **MgSO₄ · 7H₂O**
  - 0.5 g
- **K₂HPO₄**
  - 1.0 g
- **FeSO₄**
  - 0.01 g
- **Agar**
  - 20 g
- **Distilled water to make**
  - 1000 ml

**Richard’s Agar**

- **Sucrose**
  - 50 g
- **Potassium nitrate**
  - 10 g
- **Potassium dihydrogen phosphate**
  - 5 g
<table>
<thead>
<tr>
<th><strong>Chemical</strong></th>
<th><strong>Amount</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Medium A** (modified Fries, 1965)

<table>
<thead>
<tr>
<th><strong>Chemical</strong></th>
<th><strong>Amount</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10 g</td>
</tr>
<tr>
<td>Asparagine</td>
<td>1 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1 g</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂·12H₂O</td>
<td>2.25 g</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>0.25 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.1 g</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.41 g</td>
</tr>
<tr>
<td>Ferricitrate</td>
<td>5.31 mg</td>
</tr>
<tr>
<td>Citric acid</td>
<td>5.31 mg</td>
</tr>
<tr>
<td>Na₂SO₄·3H₂O</td>
<td>4.43 mg</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>4.05 mg</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

The salts calculated for 1000 ml, were dissolved in 800 ml distilled water and sterilized 20 ml per flask. Carbon and nitrogen sources were dissolved in 200 ml distilled water and 5 ml of this solution added after proper sterilization, aseptically per flask.
**1/16 M Sorensen's phosphate buffers**

### Composition of buffer solution

<table>
<thead>
<tr>
<th>ml 1/16 M ( \text{KH}_2\text{PO}_4 )</th>
<th>ml 1/10 N HCl</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>21</td>
<td>2.0</td>
</tr>
<tr>
<td>145</td>
<td>5</td>
<td>3.0</td>
</tr>
<tr>
<td>147</td>
<td>3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>ml 1/15 M ( \text{KH}_2\text{PO}_4 )</th>
<th>ml 1/15 M ( \text{Na}_2\text{HPO}_4 )</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>149.5</td>
<td>0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>131.5</td>
<td>18.5</td>
<td>8.0</td>
</tr>
<tr>
<td>55</td>
<td>95</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>147</td>
<td>8.0</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>ml 1/15 M ( \text{K}_3\text{PO}_4 )</th>
<th>ml 1/15 M ( \text{Na}_2\text{HPO}_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>90</td>
</tr>
</tbody>
</table>
Chemical methods

Chemical composition of a number of soil samples and plant materials were determined during the present investigations. The methods of estimation followed are recorded below. Methods for the preparation of hydrochloric acid extract; estimation of loss on ignition; calcium, magnesium, potassium and phosphorus (reported as P₂O₅) were modified to suit the present work.

Collection and preparation of soil samples:

Composite soil samples were collected from top three inch depth of the various morel bearing localities and other areas. The soil samples were air dried, powdered and passed through a 60 mesh sieve. Samples from burnt sites were prepared without drying. The procedures adopted were the same as prescribed by the Association of Official Agricultural Chemists (A.O.A.C., 1945, p. 1).

Moisture: (Piper, 1966, p. 69)

The moisture content was determined by heating 10 g of air-dry soil in an oven at 105°C for 12-16 hours. It was cooled in a desiccator and weighed. The loss in weight gave the moisture content.

\[ \text{Percentage of moisture} = \frac{\text{Loss in weight}}{\text{Total weight}} \times 100 \]

Loss on Ignition: (Piper, 1966, p. 59)

Ten grams of oven-dried soil was weighed in a
Silica crucible and ignited in a muffle furnace at 500-550°C for half an hour. Cooled in a desiccator and weighed. The procedure was repeated a number of times till constant weight was obtained.

**Percent loss on ignition = Difference in weight x 10.**

**Preparation of hydrochloric acid extract (Hendrick, 1931).**

The procedure recommended by the Agricultural Educational Association was followed with a slight modification. Ignited soil sample was taken instead of air-dry sample to avoid interference of organic matter in the different determinations. Ten grams of the ignited soil sample were heated to boiling with 100 ml of concentrated hydrochloric acid on a sand bath and the boiling was continued for an hour. Thereafter it was filtered in a 500 ml volumetric flask and was made up to the mark with distilled water.

**Acid insoluble:**

The insoluble residue left after the preparation of hydrochloric acid extract was dried in an hot air oven at 100°C for 12 hours. Thereafter it was ignited, muffled, cooled and weighed:

**Percentage of acid insoluble = Difference in weight x 10.**

**Sesquioxide (Wright, 1934).**

Hundred ml of hydrochloric acid extract was taken. The sesquioxide was precipitated by adding ammonium
chloride (5 g approximately) and an excess of dilute ammonia (1:1). The precipitate was filtered and washed free from chloride with distilled water. The filtrate and washings were preserved for the determination of calcium and magnesium. The precipitate was dried, ignited, muffled and weighed:

\[
\text{Percentage of sesquioxide (Fe}_2\text{O}_3 \times \text{Weight of the precipitate} = x 5 \times 10.
\]

**Iron oxide**: (Wright, 1934)

The sesquioxide precipitate washed free from chloride was treated with zinc dust (5 g approx.) and 30% sulphuric acid (60 ml approx.). The flask was corked with a rubber stopper fitted with a Bunsen valve to maintain an atmosphere of hydrogen gas and kept overnight for the reduction of ferric iron to ferrous stage. After filtration, solution was titrated with 0.1 N KMnO\(_4\) solution:

\[
1 \text{ ml of 0.1N KMnO}_4 = 0.008 \text{ g Fe}_2\text{O}_3. \text{ Hence percentage of Fe}_2\text{O}_3 = \text{No. of ml of 0.1N KMnO}_4 \times 5 \times 10 = 0.008
\]

**Calcium oxide**: (Piper, 1966, p. 147)

The filtrate and washings from the sesquioxide determination were concentrated to 100 ml and 1 ml of glacial acetic acid was added and boiled; calcium was precipitated as calcium oxalate with 10 percent ammonium oxalate solution. The precipitate was kept overnight. Next day it was filtered and washed free from oxalate ions with hot distilled water. The precipitate was dissolved in dilute H\(_2\)SO\(_4\) (1:4) (50 ml approx.) and titrated against 0.1N KMnO\(_4\) solution at 70°.
1 ml of 0.1N KMnO₄ = 0.0028 g of CaO

Percentage of CaO = \( \frac{\text{No. of ml of 0.1N KMnO₄ used} \times 0.0028}{5 \times 10} \)

Magnesium oxide: (Piper, 1966, p. 148)

The combined filtrate and washings obtained from calcium determinations were concentrated to 100 ml on a sand bath. Magnesium was precipitated as magnesium ammonium phosphate (\( \text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O} \)) by adding an excess of saturated solution of dibasic ammonium phosphate (50 ml approx.) and an excess of dilute ammonia (1:1) (30 ml approx.). The precipitate was allowed to stand overnight and filtered and washed several times with dilute ammonia (1:9). The precipitate was dried and ignited on burner. The samples were ignited in an electric muffle furnace at a bright red heat at about 700° for one hour, cooled and weighed as \( \text{Mg}_2\text{P}_2\text{O}_7 \):

\[
\text{Weight of MgO} = \text{Weight of precipitate} \times 0.362
\]

\[
\text{Percentage of MgO} = \text{Weight of precipitate} \times 0.362 \times 5 \times 10
\]

Potassium oxide: (Jackson, 1958, p. 125)

Fifty ml of hydrochloric acid extract was evaporated to dryness on a water bath. When cold 1 ml of glacial acetic acid and 10 ml of sodium chloride were added. Subsequently 10 ml of 35 per cent sodium nitrite was added and stirred well. Then an excess of sodium cobaltinitrite (50 ml approx.) was added quickly to precipitate potassium as \( \text{K}_2\text{NaCo(NO}_2\text{)}_6 \). The precipitate
was allowed to stand overnight, afterwards was filtered through a Crucible crucible and washed three to four times with 36 per cent alcohol.

The Crucible crucible with the precipitate was transferred to the original beaker in which the precipitation was carried out and 5 ml of dilute sulphuric acid (1:20) were added to it. Then a known volume (20 ml) of 0.1N KMnO₄ was added to it, and warmed gently. A known volume of 0.05N oxalic acid (20 ml) was added and heated till all the oxides of manganese dissolved. The excess of oxalic acid was titrated with 0.05N KMnO₄. The difference between the total amount of permanganate and oxalic acid added corresponds to the amount of KMnO₄ consumed to oxidise the cobaltinitrite:

\[ 1 \text{ ml of 0.1N KMnO₄} = 0.000856 \text{ g of K₂O} \]

Phosphorus (estimated as P₂O₅) (Piper, 1966, p 150)

Fifty ml hydrochloric acid extract was evaporated to dryness on a waterbath. The residue was then digested with 20 ml of concentrated nitric acid and filtered when cold. The volume of the filtrate was made to 120 ml approximately and again evaporated to dryness. Residue was dissolved in 30 ml of Acid reagent II (14 ml of concentrated sulphuric acid mixed with 60 ml of water and then added gently to 260 ml of concentrated nitric acid) and heated to boiling on a burner. Then it was filtered and filtrate was brought to boiling again. Filtrate was removed from
the flame, stirred for some time and 30 ml of Lorenz reagent I (sulphate-molybdate acid) added to it. After allowing it to stand for 2-5 minutes, it was stirred again and left overnight. Subsequently it was filtered and washed free from nitric acid first with 1 per cent ammonium nitrate solution and finally with 1 per cent potassium nitrate solution. An excess of a standard sodium hydroxide (0.1N) solution was added to dissolve the precipitate of ammonium phospho-molybdate. The unused sodium hydroxide was back titrated against 0.1N HCl using phenolphthalein as an indicator:

\[ 1 \text{ ml of 0.1N NaOH solution used} = 0.000309 \times P_{2O_5} \]

\[ \text{Percentage of } P_{2O_5} = \frac{\text{No. of ml of 0.1N NaOH used}}{0.000309} \times 10 \times 5. \]

Available phosphate (Dyer, 1934)

Ten grams of soil was taken and to it 100 ml of 2 per cent citric acid was added and left for seven days. Thereafter it was filtered and evaporated to dryness. The residue was dissolved in hydrochloric acid and again evaporated to dryness. Sufficient concentrated nitric acid was added and the contents were digested again. The insoluble portion was filtered off and the filtrate phosphate was precipitated and estimated as in the case of total $P_{2O_5}$.

Estimation of organic matter (Jackson, 1958, p.222)

Air-dry soil (0.2 - 1.0 g) having been passed through a 100 mesh sieve was weighed and transferred to a
250 ml Berzelius beaker. Ten ml of water was added to the soil followed by the addition of 8 ml of 0.1N HCl. The suspension was stirred to facilitate reaction and finally warmed on a hot plate for an hour. A drop of salt-free bromocresol green indicator was added. If the indicator turned blue additional 0.1NHC1 was added and digestion continued until the pH remained below 5.8. The sample was thus made carbonate-free for organic matter oxidation.

To the soil suspension 10 ml of 30 percent H2O2 was added and the beaker covered with a tightly fitting watch glass and the suspension digested on an electric hot plate, carefully regulated to avoid frothing over. After all the peroxide is decomposed and the solution has evaporated to volume of approximately 5 ml, 5 ml additional 30 per cent H2O2 was added to rinse down the sides of the beaker. The digestion is continued until all the peroxide is decomposed. In case of soils with high organic matter content further additions of H2O2 were found necessary.

After completing the digestion the contents of the beaker were transferred to a 100 ml centrifuge tube into which 5 ml of the 10 per cent (NH4)2CO3 solution had been previously placed, a policeman being used to complete the transfer. Finally the suspension in the tube was mixed with a strong jet of water and the tube set aside for the soil to flocculate. After flocculation has occurred the suspension was centrifuged until the supernatant liquid was entirely clear. The clear liquid was
decanted into the original beaker. Centrifuge tube was transferred to a drying oven and drying continued at 110° for 8 hours or overnight. The tube was cooled in a desiccator and net oven dry weight of the sample determined. The percentage of the organic matter was calculated as follows:

\[
\text{Per cent organic matter} = \frac{\text{Difference in weight}}{\text{Wt. of the original sample}} \times 100
\]

**Estimation of total nitrogen** (Treadwell and Hall, 1947)

The total nitrogen of sample was estimated by the salicylic acid reduction method.

A weighed amount of the sample was added in cold to 30 ml of concentrated sulphuric acid containing 1 g of salicylic acid in a Kjeldahl flask. The flask was occasionally shaken. The treatment binds the nitrate nitrogen into organic combination (5-nitrosalicylic acid). After 30 minutes, 5 g of sodium thiosulphate was added to reduce the nitro group to amino-salicylic acid.

The mixture was then carefully heated until the frothing subsided. After cooling 5 g of potassium sulphate and few crystals of copper sulphate were added. The mixture was then digested until the solution became clear. The digestion was again continued for an additional 20-30 minutes to ensure complete oxidation. After cooling distilled water was added and this solution containing all nitrogen as ammonium sulphate was filtered and made upto a measured volume. An aliquot portion of this solution was now distilled with 50 ml of 40 per cent sodium hydroxide.
The ammonia was absorbed in a known excess of standard sulphuric acid (N/50). The unchanged acid was titrated against N/50 sodium hydroxide solution using mixed indicator (methylene blue and methyl red). The total nitrogen was calculated by using the factor:

\[ 1 \text{ ml of } 0.1N \text{ H}_2\text{SO}_4 = 0.0014 \text{ g N} \]

**pH determination**

Philips pH meter was used for the determinations. Buffers of pH 4.7 and 9.18 obtained by using 0.01M acetic acid - 0.01M sodium acetate (4.7) and 0.05M - borax (9.18) were used for standardising and as reference. Soil samples were mixed with water in the ratio of 1:5 (w/v) and shaken for half an hour. Immediately thereafter the pH was determined.

**Analysis of plant material**

Morel fruits and mycelial material was dried in an incubator with air circulating arrangements at 40°C. Subsequently it was crushed with a clean pestle and mortar and passed through a 60 mesh sieve. Ten grams of the material were taken in a 50 ml capacity vitreous silica crucible. Two to three drops of olive oil were added to make it cohesive. Initially the crucible with the material was ignited carefully on a low bunsen flame to avoid spurtting. Later on it was muffled at 550°C for an hour. Ignition at this temperature was carried out till constant weight was obtained. The per cent age of ash was determined.

The methods employed for various estimations were same as described earlier for soil analysis.