Quantitative analysis of total nitrogen, phosphorus, potassium, calcium, magnesium, crude protein% and the caloric values has been carried out on some palatable grasses of Garhwal Himalaya are infected with Phyllachora sp., causing black leaf spot disease. These estimates have duly been compared with those of the healthy specimens growing under similar ecological conditions. It has been observed that all the studied parameters show a decline in their values for the diseased plants, the extent of decline; of course depends upon the plant species and the severity of infection. This decrease in nutrients has been attributed to reduce the rate of photosynthesis and a subsequent hindrance to other related metabolic activities.

Key words: Phyllachora sp., Garhwal, Himalaya, nutrients, palatable.
conditions have naturally produced rich as well as diverse vegetation.

II- Host-parasite relationship:
Among many parasites of the cereals, grasses and numerous other non-economic plants, the black leaf spot disease caused by various species of *Phyllachora* (Ascomycetes) have attracted much attention over the past decades. These are widely prevalent in the hilly region of India; where lofty mountain ranges associated with thick forests and high rainfall provide optimum conditions for the growth and development of the species. Black leaf spots are not pustules but small black paste-like appearance. 'Black leaf spot or Tar spot' caused by species *Phyllachora*, appears as small black spots on of dorsal surface of the leaves and is a common pathogen on many native grasses of grazinglands of Uttarakhand Himalaya. The causal fungus is a biotroph, feeding on living cells of the host and causing little necrosis or chlorosis of leaves, except at the immediate infection site. Infected grasses are not killed outright and leaves remain green through most of the growing season. The relatively mild symptomology of these gramineous host-parasite relationship has not been studied extensively (Gabel 1989, Mehta 1992).

Methods:
During the course of the present investigation the plants growing in different parts of the Garhwal Himalaya were surveyed widely for the presence of black leaf spot disease. The initiation (September) and the time of severity (November) of the disease were noted. During the investigation the diseased and healthy specimens were collected from the humid and shady locality of this region. The diseased and healthy plant leaf materials were collected separately in polythene bags and brought to the laboratory for the estimation of nutrient contents.

The samples were dried and analyzed for nutrient content i.e., nitrogen, phosphorous and potassium as follows:

Aliquots of dried leaf materials were homogenized to powder. Total nitrogen percentage was determined by colorimetric techniques (Shell and Shell 1965). Nessler's reagent was added to digested solution (conc. \( H_2SO_4 \), catalyst) of the leaf sample and the resulting yellow solution was measured in a Spekol colorimeter at 410 nm. For phosphorous, the ash solution was prepared by wet ashing procedure using nitric, perchloric acid and sulphuric acid (Piper 1944). Known quantity of ammonium molybdate was added to develop blue colour and percent transmission was measured at 660nm, and the amount was calculated from calibration curve (Jackson 1962). Potassium was estimated by Flame Photometer as described by Jackson 1967.

For the estimation of Ca and Mg percentage, samples were prepared by digestion of powdered samples in HCl and Ca was then determined using a Flame Photometer and Mg% by using an Atomic Absorption Spectrophotometer. Crude protein (\%) was obtained by multiplying total nitrogen percentage by a factor of 6.25, as given by Coombs et al. (1985).

The calorific values were determined from samples collected from different localities of Garhwal. With the help of powdered materials, three pellets (Cal gm-1) were prepared for each sample and kept in desicator for drying. The pellets were then ignited in Oxygen Bomb Colorimeter with oxygen pressure 13-15 atm, following the procedure suggested by Leath (1968). The calorific value per gram dry weight was calculated by the following formula (Parr instrument Company manual 130, 1968):

\[
Hg = tw - e_1 - e_2 - e_3 \times Cal.gm^{-2} \\
\text{m}
\]

Where \( Hg \) = gross heat of combustion

\( t \) = rise in temperature (°c)

\( w \) = water equivalent of Colorimeter (Cal)

\( e_1 \) = acid correction of HNO\(_3\) (Cal)

\( e_2 \) = acid correction of H\(_2\)SO\(_4\) (Cal)

\( e_3 \) = fuse wire correction (Cal)

Acid corrections have been known to be negligible hence no corrections have been made in the present study. The water equivalent (w) of
the Colorimeter was determined by performing blank experiment using benzoic acid and calculation by using the formula (Parr Instrument Company 130, 1968).

\[ w = \frac{Hm - e \times e \times \text{Cal.gm}^{-1}}{t} \]

Where \( m \) = mass of benzoic acid
\( H \) = calorific values of benzoic acid (Cal.gm\(^{-1}\))
\( e \) = correction for HNO\(_3\) (m\(^{-1}\) = Cal)
\( t \) = rise in temperature (°C)

**RESULTS AND DISCUSSION**

In the black leaf spot disease, symptoms were appeared on leaves and stems in the earlier days of September, when a countable number of spots were noticed recorded as a initiation phase of the disease. In the later days of October and end of November the extension of spots were recorded numerous in number and covered almost (60-75 %) part of the leaf area and 40-45% of stem area, this phase denoted as a time of severity.

The severity level and degree of infection recorded from the area of leaves and stems infected by the pathogen manually i.e., first counted the number of spots appeared on the infected material directly, on the basis of first recorded observations, the ratio of infected area (black coverage) and photosynthetically active area (green part/area) were calculated. The mode of infection varied on the selected grass species depends on their time of initiation and severity, leaf area and structure. For the better growth aspects of pathogen, the winter season, especially the month from September-November were recorded most convenient, when in the temperate regions having moderate climatic conditions. The temperature, moisture and humidity in atmosphere and soil remained optimum for the growth of pathogen.
Microclimatic conditions also favored to initiate and extend it to the severity level of infection. Most of the palatable fodder grass species flourished under the chir-pine canopy, so the atmospheric as well as the soil conditions were ideal for the growth and developmental activities of the pathogen causing back leaf spot disease.

In the present investigation (Table 1) healthy grass species had more nitrogen than the diseased. Some grasses viz. *Arthraxon lanceolatus* (2.64%), *Arundinella setosa* (2.37%), *Eragrostis nigra* (2.17%) and *Cynodon dactylon* (1.97%) have shown a decline in nitrogen contents while remaining species were only slightly affected. The significantly higher amount of nitrogen in healthy plants than the diseased may be associated with higher rate of photosynthesis. The nitrogen amount of an ecosystem may be relatively stable, or it may be changing in quality, depending on the net gain and loss of nitrogen by various input and output processes (Collier et al. 1973). The loss of nitrogen through volatilization from infected grasses varies with the degree of infection. When the infection does not cover all the leaf area, the loss seldom exceeds 30% of the total nitrogen; however, if infection covers whole leaf area completely, the loss may exceed 75% of the total nitrogen in leaf material.

The percentage phosphorous concentration ranged 0.27% (*Agrostis pilisula*) to 0.55% (*Eulalia mollis*) in healthy grasses and between 0.17% (*Agrostis pilisula* and *Arundinella setosa*) and 0.35% (*Eragrostis nigra*) for diseased. The loss of phosphorous contents in percent was recorded significantly different between diseased and healthy plants respectively i.e., 0.17-0.36% (*Arundinella setosa*), 0.27-0.46% (*Cynodon dactylon*) and 0.18-0.55% (*Eulalia mollis*).

The percentage of potassium was recorded highest in healthy grass 1.85% (*Agropyron longearistatum*). Diseased plants showed a decreasing trend for all the fodder grass species and it ranged between 0.23% (*Eulalia mollis*) to 0.68% (*Agrostis pilisula*). More amount of K% in green leaves as compared to older or infected (non photosynthetic) may be because of its depletion with the advancement of disease infection. Billore and Mall (1976) also recorded the abundance of K% in living tissue where as White (1973) observed its loss on drying/dying and infected ones. The calcium and magnesium percentage values ranged from 0.39% (*Eragrostis nigra*) to 1.59% (*Arthraxon lanceolatus*), and 0.03% (*Agropyron longearistatum*) to 1.31% (*Eulalia mollis*) for healthy, respectively. In infected grass species both the elements were ranged from 0.14% (*Arthraxon lanceolatus*) to 0.53% (*Arundinella setosa*), and 0.2% (*Agropyron longearistatum*) to 0.82% (*Eragrostis nigra*), respectively.

The percentage of crude protein contents (CP %) were severely affected by the pathogen, were recorded for different palatable fodder grasses. The maximum variation in crude protein contents in diseased and healthy plants were recorded, respectively, 6.937% and 16.50% for *Arthraxon lanceolatus*, 7.75% and 14.81% for *Cynodon dactylon* and followed by 6.25% and 10.937% for *Agrostis pilisula*.

The amount of CP% was also varied relative to the nitrogen contents. The average values of CP% (Table 1) show comparatively higher amounts in healthy plants than those in the diseased plants. Several lines of evidence indicate that there is an increase in proteins in the diseased plants in earlier stage of infection, which may gradually reduce with increase in pathogenesis (Goodman et al. 1967). For grasses and herbaceous plants, there is a distinct dependence of caloric values on climatic conditions, the availability of water and the concentration of dissolved salt in the soil (Casper 1975). It is also observed that there is considerable variation of caloric values between the individual components of plant species. In this regards our findings coincide with the finding of Runge (1971), Larcher et al. (1973) and Casper (1975, 1977). It was further observed that there is a remarkable difference in the energy values of the healthy and diseased
plants, ranging from 4068-4335 Cal.gm⁻¹ and 3900-4105 Cal.gm⁻¹ for *Arundinella setosa* and *Eragrostis atrovirens*, respectively. However, the values are not constant for all the plants but differ significantly in different species depending upon the mode and degree of infection.

A number of studies have indicated that plants with the greatest growth ratio are those which allocate the highest proportion of their photosynthetically fixed carbon to the synthesis of additional photosynthetic tissues (Mooney 1972, Johnson and Tieszen 1976). There is an effect on the host, however because infected leaves have less surface area available for photosynthetic activity. Rey and Garnett (1984) showed reduction in net photosynthesis when over 25% of *Panicum maximum* Jacq. leaf surfaces were infected with *Phyllachora paspalicola* P. Henn. Arrangement and morphology of chloroplast was changed and there was a decline in chloroplast content.

The decline in the percentage of N, P, K, Ca, Mg, CP and calorific values(Cal.gm⁻¹) may be owing to withering or leaching of the respective elements by decreased photosynthetic rate, resulting due to loss of chlorophyll by the pathogen *Phyllachora* sp. Maximum percentage values of nitrogen was recorded for the matured and healthy stage of plants. These values decrease in the later growing stage and in diseased plants, especially the leaves and hence reduced the mineral status, and the transpiration rate besides drastically affecting the photosynthetic rate. This decreased photosynthetic efficiency, coupled with the other metabolic hindrances, alter the growth rate of the infected plants. These entire disturbances in the physiological activities of the plants lead to the decline in nutrient contents, as exhibited during the present investigation.

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