Effect of different levels of N, P, and K on downy mildew 
(Peronospora plantaginis) and seed yield of isabgol (Plantago ovata)

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Abstract
The study showed that application of different doses of inorganic nutrients had considerable influence on growth, yield and downy mildew interaction in isabgol (Plantago ovata). The study included three levels of nitrogen (0, 30, 60 kg N/ha), two levels of phosphorous (0, 30 kg P/ha) and two levels of potash (0, 40 kg K/ha) for two consecutive years. Highest N supply (60 kg/ha) caused more than 65% increase in disease severity compared to control. However, application of 40 kg/ha K reduced percent disease index (PDI) by more than 10% compared to 0 kg K/ha treatment. Seed yield was influenced by N but not P or K. The highest seed yield was obtained from 60 kg/ha N during both the years. Total sugar concentration in leaf tissue increased with the application of N, but decreased phenol and OD phenol concentrations. On the other hand growth of the plant was positively influenced by N. As a result, leaf area index (LAI) registered 40.91% and 51.01% increase compared to control due to 30 and 60 kg/ha N application. Leaf chlorophyll content and net photosynthesis also increased in the N treated plants. Seed yield and disease reaction in different fertiliser treatments have been discussed in the light of different physiological and biochemical parameters studied.

Keywords: Peronospora plantaginis; yield loss; resistance determinant; high sugar disease.

1. Introduction
India has a long tradition of production and use of medicinal plants for primary healthcare. The country earns approximately 90 million US$ from export of medicinal plants and their products. Isabgol (Plantago ovata Forsk.) is a major contributor in the export earning of the country. Isabgol husk (seed epidermis), traditionally used against constipation, diarrhoea, intestinal irritation, etc. is a very good source of dietary fibre and has hypocholesterolemic activity (Kawatra et al., 1990). Recently, it is increasingly being used as food additive in several processed materials like cookies, ice-cream, bread, etc. (Pflaumer et al., 1990; Trautwein et al., 2000). However, downy mildew (Peronospora plantaginis), which infects foliar as well as floral parts (Mandal and Geetha, 2001), is one of the major constraints in cultivation of the crop successfully. It appears as frost-like downy growth on the lower side of the leaves with corresponding yellowing on the upper leaf surface. Under severe conditions, pathogen growth is visible on both the leaf surfaces. As the lesion ages, the leaf dries up causing loss in active photosynthetic area.
The crop has traditionally been grown in the drier areas of Gujarat, western India, but recently increasing demand has prompted extending its cultivation into the non-traditional areas of Rajasthan and Madhya Pradesh. Although, Kalyansundaram et al. (1982) has reported its low nutrient requirement, different levels of nitrogen (N), phosphorous (P) and potash (K) have resulted in varied yield responses (Randhawa et al., 1978; Ramesh et al., 1989; Intodia and Tomar, 1998). Farmers also have started using higher N, P, and K inputs, especially in Rajasthan.

Application of inorganic N fertilisers promotes growth as well as it increases susceptibility to foliar diseases causing starvation of nutrients in several crops (Develash and Sugha, 1974; Bains and Jhooty, 1978; Doshi and Thakore, 1995). However, in isabgol, the effect of macro-nutrients on downy mildew development is only tangentially known (Rathore and Chandawat, 2003). Increasing demand of the crop, consequent expanding cultivation coupled with use of higher inputs by the farmers may lead to an undesirable condition for isabgol farming particularly in the absence of resistant cultivars available to the farmers. Hence, the present experiment was conducted to study the effect of different doses of N, P, and K on downy mildew severity and seed yield as well as to understand the biochemical basis of differential disease reaction and physiological reasons for variation in yield due to variable nutrient doses.

2. Materials and methods
2.1. Experimental conditions
Field experiments were conducted at the research farm of National Research Centre for Medicinal and Aromatic Plants, Boriavi, Anand, Gujarat, India (73° E, 22.5° N) following factorial randomised complete block design in the winter (Nov–Mar) during 2002-03 and 2003-04. The land was exhausted by growing sorghum (Sorghum vulgare) as previous crop, resulting in available N, P, and K of 200.70–249.37 kg/ha, 1.79–2.53 kg/ha and 48.50–52.80 kg/ha, respectively prior to starting of the experiment. A highly downy mildew susceptible cultivar of isabgol, cv. Niharika, was grown for this study. Weather conditions (temperature and rainfall) during the experimentation are presented in Fig. 1.

2.2. Treatments
Different levels of N, P, and K (kg/ha) were applied: N (0, 30, 60), P (0, 30) and K (0, 40): using urea, single super phosphate, and muriate of potash, respectively. To minimise fertiliser movement from one plot to another, irrigation was applied with very slow rate so that flooding and cross contamination between the plots does not occur and individual treatments were applied in big size plots (5 x 4 m) and data were recorded leaving wide (1.2 m) border rows. Three replications were maintained for each treatment. General crop production technology (application of irrigation thrice, one weeding and one hoeing) was followed (Maiti and Mandal, 2000).

Paired block was maintained for each treatment – one with repeated fungicide (0.2% of commercial formulation of metalaxyl and mancozeb in 8:64 combination) treatment to keep the crop downy mildew free, another as check to assess the yield loss due to downy mildew.

2.3. Disease severity assessment
The unsprayed block was examined at 20-day intervals between 20 and 80 days after sowing (DAS) to assess the downy mildew severity. It was measured by calculating
percent disease index (PDI). At least six plants were randomly selected, all the leaves were rated following a 0–4 scale (0, no disease; 1, 1–25% leaf area affected; 2, 26–50% leaf area affected; 3, 51–75% leaf area affected and 4, >75% leaf area affected; Mandal et al., 2007). PDI was calculated as follows:

$$\text{PDI} = \frac{\sum \text{All ratings} \times 100}{\text{Number of leaves} \times 4 \text{ (maximum rating)}}$$

2.4. Estimation of sugar, phenol, and Ortho-Dihydric (OD) phenol contents in leaf

It was evident from first year’s results that different nutrients had significant effects on downy mildew severity as well as crop growth and yield. Hence, during the second year, we analysed the plant samples for different biochemical and physiological parameters to understand the underlying mechanisms.

Fully expanded leaf samples (fourth to sixth leaf from apex) were randomly collected from each plot ($r=3$). The samples were weighed and kept at -70°C until used. For biochemical analysis, the samples were extracted in hot 80% ethanol and aqueous phase of this extract was used for biochemical analysis. Total soluble sugar was estimated using anthrone reagent (Sadasivam and Manickam, 1997). In 1 ml of diluted plant extract 4 ml of freshly prepared anthrone reagent (2 g of anthrone dissolved in 1 lt of concentrated sulphuric acid) was added and heated on a boiling water bath for 10 mins. The tubes were removed, cooled to room temperature and absorbance of the content was measured at 625 nm in a spectrophotometer (Cary 300 Bio, Varian, Mulgrave, Australia). Amount of total sugar present in the sample was calculated from a standard curve drawn from variable amount of glucose. Total phenol was estimated by Folin-phenol reagent (Bray and Thrope, 1954). To 1 ml diluted plant extract equal volume of Folin-phenol reagent (E-Merck India, Mumbai, India) was added followed by 2 ml of 20% aqueous sodium carbonate. The content was mixed thoroughly and incubated at room temperature for 1 hr. Absorbance of the blue coloured solution was measured at 650 nm and total phenol was calculated from a standard curve developed using catechol as the standard. Arnow’s method was followed to determine OD phenol (Arnow, 1937). One millilitre of diluted plant extract was taken in a test tube and equal volume of 0.5 N hydrochloric acid was added to it followed by 1 ml of Arnow’s reagent (10 g sodium nitrite and 10 g sodium molybdate dissolved in 100 ml of distilled water) and 2 ml of 1 N aqueous sodium hydroxide. Absorbance was measured spectrophotometrically at 515 nm. OD phenol in the sample was estimated from a standard curve prepared with catechol as a standard.

2.5. Determination of leaf area index (LAI)

LAI was recorded using plant canopy analyzer (LAI-2000, LI-COR Inc, Lincoln, USA) following all the precautions to avoid errors in measurement. The measurements were taken inside the canopy of unsprayed block during 75-80 DAS.

2.6. Measurement of net photosynthetic rate

Net photosynthetic rate ($Pn$) was measured in three categories of leaves: (a) uninfected leaves grouped as healthy; downy mildew infected leaves showing (b) slight and (c) severe loss of chlorophyll. Leaf samples ($n=5$, $r=3$) were marked and $Pn$ was measured using a portable open infrared gas analyser (LI-6400, LI-COR Inc., Lincoln,
USA). Measurements were made using a standard leaf chamber (2×3 cm) having transparent top.

2.7. Estimation of chlorophyll

Following photosynthetic measurement leaf samples were utilised for chlorophyll estimation. Samples belonging to three symptomatological groups from different treatments \((r=3)\) were collected and extracted in 7–8 ml of dimethyl sulphoxide (DMSO) for 1 hr at 65°C under darkness. At the end the green solution was decanted in a measuring cylinder and volume was made to 10 ml with DMSO. Absorbance was taken at 663 and 645 nm in a spectrophotometer (Cary 300 Bio, Varian, Mulgrave, Australia). Total chlorophyll content was estimated spectrophotometrically (Arnon, 1949).

2.8. Seed yield and loss estimation

The crop was harvested at maturity, threshed, cleaned and bagged manually. The yield was calculated and expressed in kg/ha basis. Reduction in seed yield in the control block in comparison to fungicide block was calculated and expressed in percent basis.

2.9. Statistical analysis

Statistical analysis was carried out using the statistical package MSTAT-C version 1.4 (Crop and Soil Science Division, Michigan State University, USA). Square root transformation of the PDI values (ranging between 8.31 and 19.72) were done (Gomez and Gomez 1984). Transformed data were used for ANOVA. Least significant differences (lsd) \((P=0.05)\) were compared between two treatment means. Simple correlation between two data sets was calculated in MS Excel spread sheet.

3. Results

3.1. Disease severity

Crop was monitored every day to detect disease incidence. The disease could not be detected before 30 days of crop age; however, it spread considerably between 60 and 80 DASs. N and K significantly influenced downy mildew severity during both the years (Table 1), with N increasing disease severity. During 2002-03, zero N showed least disease severity with 10.26 PDI at 60 DAS. Disease severity increased with 30 and 60 kg N/ha (PDI 12.18 and 13.00, respectively), but difference between these two nitrogen doses was non-significant. At 80 DAS, disease severity gradually increased with increasing N dose.

K, on the other hand, reduced the downy mildew severity (Table 1). During first year of study, at 60 DAS, application of K did not have a significant effect on PDI compared to zero K; however, at 80 DAS, application of 40 kg K/ha reduced the PDI to 15.14 compared to 17.00 in the control. A similar trend was observed during second year (2003-2004) of the experiment. At 60 DAS, the lowest PDI of 8.31 was recorded in 0 kg N/ha and the highest was in 60 kg N/ha (16.84) followed by 30 kg N/ha (13.80). At 80 DAS, disease severity gradually increased with increasing N dose.
P did not have any significant effect on downy mildew severity during both the years. All interaction effects were also found statistically non-significant (data not shown).

3.2. Leaf bio-constituents

Total soluble sugar and phenol contents in isabgol leaves were influenced by different N, P, and K treatments. Total sugar content increased with the increase in N doses (Fig. 2). In general, sugar content decreased during 80 DAS compared to 60 DAS. Lowest total sugar values were found in plants receiving zero N (114.69 and 98.50 mg/g at 60 and 80 DASs, respectively) while highest sugar values (179.01 and 127.95 mg/g at 60 and 80 DASs, respectively) was associated with highest level of N. Application of 30 kg N/ha resulted an intermediate sugar concentration of 152.55 mg/g leaf at 60 DAS while, it was 103.95 mg/g at 80 DAS. However, P or K had no significant effect on total soluble sugar content at 60 and 80 DAS.

Total phenol content, on the other hand, was influenced by both N and K (Fig. 3). Application of N decreased total phenol content of isabgol leaves. Application of 0, 30 and 60 kg N/ha resulted in 26.97, 20.18 and 18.35 mg phenol during 60 DAS and 25.07, 18.10 and 16.00 mg phenol during 80 DAS, respectively, on a dry weight basis (mg/g). The opposite trend was observed with K. Total soluble phenol was at its highest in plants receiving 40 kg K/ha (22.71 and 20.64 mg/g leaf during 60 and 80 DAS, respectively) compared to 20.96 and 18.81 mg phenol/g leaf during 60 and 80 DAS, respectively, in control.

OD phenol concentration in isabgol leaves also followed the similar trend as that of total phenol. Application of inorganic N gradually reduced the OD phenol concentration (Fig. 4). Lowest OD phenol contents (3.89 and 1.86 mg/g leaf during 60 and 80 DASs) were noted with 60 kg N/ha, while the highest concentration was in zero N application (5.48 and 3.35 mg/g leaf at 60 and 80 DASs, respectively). At 80 DAS, application of K resulted in higher OD phenol concentration (2.68 mg/g leaf) compared to control.

3.3. LAI

LAI was positively influenced by N application and better vegetative growth was observed with N application. Highest LAI (2.99) was observed in 60 kg N/ha while the lowest (1.98) was in the zero N treatment. Application of 30 kg N/ha resulted in an LAI of 2.79, which was significantly higher than the zero N treatment, but not different from the 60 kg N/ha treatment. Other two nutrients (P and K) and all interaction effects were non-significant on LAI.

3.4. Leaf chlorophyll content

Application of N increased total chlorophyll contents irrespective of categories of leaf. Expectedly, plants receiving same level of N had higher chlorophyll content in healthy leaves while it reduced with the increase in severity of disease. Within the same severity group, total chlorophyll contents increased with the increase in N (Fig. 5). Healthy leaves contained the most total chlorophyll at 60 kg N/ha (0.62 mg/g leaf). However, it was not statistically different from the leaves from 30 kg N (0.49 mg/g leaf). On the other hand, control plants had lowest total chlorophyll (0.47 mg/g leaf), which was not statistically different that than of the 30 kg N/ha treatment. A similar trend was observed in slightly and severely chlorotic leaf groups as well. Highest total chlorophyll in slightly chlorotic leaves was observed with 60 kg N/ha (0.44 mg/g leaf) while the
lowest one was in the 0 N treatment. In severely chlorotic leaves, control, 30 and 60 kg N had 0.20, 0.23 and 0.28 mg/g total chlorophyll, respectively. P or K did not have significant effect of leaf chlorophyll content (data not shown).

3.5. Photosynthetic rate

\( P_n \) decreased with the increase in chlorosis in plants receiving the same N dose. Increasing the N dose increased \( P_n \) within same category of leaf (Fig. 6). Healthy leaves had \( P_n \) of 17.90 \( \mu \)mol CO\(_2\)/m\(^2\)/sec in control, which increased to 21.21 \( \mu \)mol CO\(_2\)/m\(^2\)/sec and 21.84 \( \mu \)mol CO\(_2\)/m\(^2\)/sec in 30 and 60 kg N/ha, respectively. Overall, N alone, not P or K, influenced photosynthetic activity.

3.6. Seed yield

In the present study, variation between seed yield of two years was high. Non-seasonal rain towards late February and higher air temperature (Fig. 1) during anthesis and grain filling stages of the crop in 2002-2003 possibly caused lower seed yield. However, trends in treatment effects recorded during individual years remained the same.

Application of N had significant effect on seed yield of isabgol. Yield increased with the increase in the dose of N (Table 1). During the first year of study, control plot produced only 995.53 kg/ha of seed. This was the lowest compared to yields from other two doses of N. Application of 30 and 60 kg N/ha resulted 1129.36 and 1104.48 kg/ha of seed yield, respectively, being statistically not different between themselves. During 2003-2004 also, N application increased seed yield. Lowest seed yield was obtained from plots receiving zero N (2065.64 kg/ha) while highest yield of 2287.03 kg/ha was recorded in the plots receiving 60 kg/ha N.

Individual effects of P and K and other interaction effects were not statistically significant for seed yield.

3.7. Yield loss

Downy mildew resulted in yield reduction in all the treatments compared to fungicide protected block. Highest yield reduction was noticed in treatment receiving 60 kg N/ha. During 2002-2003 and 2003-2004, downy mildew in the 60 kg N/ha treatment resulted in 514.56 and 813.49 kg/ha yield reduction, respectively. The yield reduction was 31.78% during the first year and 35.57% in the next year. K application resulted in less yield loss than at zero K level. Downy mildew in the 40 kg K/ha treatment resulted in 319.97 kg/ha yield loss compared to loss of 399.35 kg/ha in zero K during 2002-2003. In the following season, losses in seed yield in treatments with and without K were 580.79 and 601.63 kg/ha, respectively. P did not have significant effect on yield loss (data not shown).

4. Discussion

The primary aim of this two-year study was to determine how N, P, and K influence downy mildew disease development under field conditions and its impact on seed yield of isabgol thereby helping cultivators to grow the crop more economically. It was observed that downy mildew severity in the field was consistently favoured by application of inorganic N. However, application of K reduced disease development to some extent.

Application of inorganic nitrogenous fertiliser has been reported to increase downy mildew disease severity in different crops (Doshi and Thakore, 1995; Develash and Sugha, 1997). In isabgol, Rathore and Chandawat (2003) have observed that
application of 60 kg N/ha significantly increased downy mildew severity. Decrease in
downy mildew incidence due to K has also been reported (Develash and Sugha, 1997;
Angadi and Vijaykumar, 2000). Our study also confirmed that downy mildew severity
increased with N application in a dose dependent manner. Correlation coefficient
between N and PDI was significant (0.974 and 0.900 during 2002-03 and 2003-04,
respectively); while it was –0.580 and –0.563 during these two years with respect to K
and disease severity.

Influence of sugar concentration on disease development varies depending upon
host-pathogen system. In a plant-biotroph interaction involving Italian millet (Setaria
italica) and rust (Uromyces setariae-italicae), higher sugar content in the host has been
found to induce susceptibility (Vidyasekaran et al., 1974). However, Lyles et al. (1959)
suggests that in wheat rust disease, varieties having higher sugar content are resistant to
infection by Puccinia graminis tritici. In the present study, total sugar of isabgol leaves
was higher in N-treated plots and correspondingly higher downy mildew severity was
also observed. There was a significant positive correlation between disease severity (PDI)
and total sugar (r = 0.585).

Phenolics – total phenol and OD phenol – are two components that induce
resistance in plants against pathogens (Vidyasekaran, 1973). Decreasing phenol content
in mustard is correlated with its increased susceptibility to white rust (Kumar et al.,
2002). In the present study, correlation coefficient between total phenol and PDI was
significant (r= –0.604). Phenol is oxidised to highly toxic OD phenol by enzymatic action
(polyphenol oxidase) and its concentration is highly correlated with low disease
susceptibility in grapes against Gloeosporium ampelophagum (Vidyasekaran, 1973). In
the present study, N resulted in reduction of total phenol as well as OD phenol
concentration in leaf tissue while K applications had opposite influence. PDI and OD
phenol had high negative correlation (r = –0.733).

Despite increased downy mildew severity, enhanced seed yield was obtained in N
treated plots. Rathore and Chandawat (2003) have also reported that seed yield of isabgol
is increased with N compared to control even though downy mildew severity is increased.
Physiological parameters like LAI, leaf chlorophyll concentration and \( Pn \) were studied to
explore the possible reasons for such conflicting results.

The positive influence of N on different physiological parameters associated to
anabolic reactions probably compensated the loss due to higher disease severity.
Application of N enhanced vegetative growth which caused 40.91% and 51.01% increase
(compared to control) in LAI with 30 and 60 kg N/ha, respectively. LAI, as a measure of
foliar growth, influences total photosynthetic area and this in turn contributes to yield
(Singh et al., 2003). Significant positive correlation (r=0.625) between LAI and seed
yield suggested that similar mechanisms were responsible for higher seed yield in the N
treated plots. It was also observed that treatments having good foliar growth retained dew
for longer period on the canopy (data not shown). LAI might have also influenced the
micro-climate around the infection site and created conducive conditions for downy
mildew to grow under shade and higher humidity. In the present experiment, LAI and
PDI had significant positive correlation (r=0.681).

In addition to LAI, leaf chlorophyll content and \( Pn \) also contributed to the
anabolic reaction, thereby influencing the crop yield. Chlorophyll contents in both
healthy and diseased leaves were higher when N was applied. Similarly, \( Pn \) also
increased with N fertilisation. Highest $Pn$ was always found to be associated with the highest N supply. Correlation coefficient between yield and $Pn$ in healthy leaves was statistically significant ($r=0.726$).

These results reflect that (i) application of inorganic N increases downy mildew severity in isabgol while K reduces it; (ii) higher susceptibility of N treated plants may be due to higher sugar and lower phenols in the leaf; (iii) N increases leaf chlorophyll content and rate of CO$_2$ assimilation in isabgol; (iv) seed yield is also increased with N application. The results also provide a scientific explanation of the farmers’ practice of using higher fertiliser inputs to increase the productivity of isabgol. However, the productivity could still be increased by following appropriate control measures for downy mildew disease. Further studies are required to ascertain the economics of such management strategies vis-à-vis quality of produce obtained from such production system. Future studies in this area may generate data for developing a working model on crop growth as well as prediction of downy mildew severity and crop yield.

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References


Table 1

<table>
<thead>
<tr>
<th>Nutrients (kg/ha)</th>
<th>Disease severity (PDI)</th>
<th>Seed yield (kg/ha)</th>
<th>Yield loss (kg/ha)</th>
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<tr>
<td></td>
<td>60 DAS</td>
<td>80 DAS</td>
<td>60 DAS</td>
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<tr>
<td>Nitrogen</td>
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<tr>
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<td>10.26</td>
<td>11.93</td>
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<tr>
<td></td>
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<td>(3.30a)</td>
<td>(3.88b)</td>
<td>(3.38b)</td>
</tr>
</tbody>
</table>

Different letters within the same column and same nutrient denote significant difference between different levels of same nutrient according to LSD test (P=0.05).

- Figures in parentheses are square root transformed values of original PDI data
- Least significance difference (P=0.05) for nitrogen and potash were 0.36 and 0.29, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 0.21 and 0.17, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 0.32 and 0.26, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 0.24 and 0.19, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 55.23 and 45.10, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 148.67 and 121.39, respectively
- Least significance difference (P=0.05) for nitrogen and potash were 208.53 and 170.26, respectively
Figures in parentheses are percent yield loss due to downy mildew compared to treated block.

Least significance difference ($P=0.05$) for nitrogen and potash were 54.08 and 44.16, respectively.

Least significance difference ($P=0.05$) for nitrogen and potash were 192.14 and 156.89, respectively.
Fig. 1 A summary of daily maximum (MAXT1 and MAXT2) and minimum (MINT1 and MINT2) temperature and rainfall (RF1) data during the study period of first and second year of experimentation.

Fig. 2 Effect of levels of nitrogen on total sugar content of isabgol leaf at: (A) 60 DAS and (B) 80 DAS. The error bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident. Bars topped by a different letter denote significant difference between the levels of nitrogen. The least significance difference ($P = 0.05$) for 60 DAS and 80 DAS were 20.24 and 15.43, respectively.
Fig. 3 Effect of levels of nitrogen and potash on total phenol content of isabgol leaf at: (A & C) 60 DAS and (B & D) 80 DAS. The error bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident. Bars topped by a different letter denote significant difference between the levels of nutrients. The least significance difference ($P = 0.05$) for 60 DAS and 80 DAS were 1.69 and 1.38, respectively.
Fig. 4 Effect of levels of nitrogen and potash on OD phenol content of isabgol leaf at: (A & C) 60 DAS and (B & D) 80 DAS. The error bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident. Bars topped by a different letter denote significant difference between the levels of nutrients. The least significance difference ($P = 0.05$) for levels of nitrogen at 60 DAS and 80 DAS were 0.43 and 0.27, respectively. While, for levels of potash at 80 DAS it was 0.22.

Fig. 5 Effect of levels of nitrogen on chlorophyll content of isabgol leaf. (A) Leaf not infected by downy mildew. (B) Leaf affected by downy mildew and showed slight chlorosis by visual observation. (C) Leaf affected by downy mildew and showed severe chlorosis by visual observation. The error bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident. Bars topped by a different letter denote significant difference between the levels of nutrients. The least significance difference ($P = 0.05$) for healthy, slightly chlorotic and severely chlorotic leaves were 0.14, 0.09 and 0.05, respectively.
Fig. 6 Effect of levels of nitrogen on rate of photosynthesis in isabgol leaf. (A) Leaf not infected by downy mildew. (B) Leaf affected by downy mildew and showed slight chlorosis by visual observation. (C) Leaf affected by downy mildew and showed severe chlorosis by visual observation. The error bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident. Bars topped by a different letter denote significant difference between the levels of nutrients. The least significance difference ($P = 0.05$) for healthy, slightly chlorotic and severely chlorotic leaves were 0.81, 0.53 and 0.64, respectively.