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Alleviating the stress of *Meloidogyne graminicola* and *Drechslera oryzae* pathogens with nitrogen application in direct seeded and transplanted rice

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ABSTRACT

Rice root-knot nematode (*Meloidogyne graminicola*) and brown spot pathogen (*Drechslera oryzae*) are two important pathogens of rice, reducing its productivity significantly. The study investigated the impact of varying nitrogen levels on the interaction between rice root-knot nematode (*M. graminicola*) and brown spot disease (*D. oryzae*) in both direct-seeded and transplanted rice. Field experiments were conducted over two years, 2019 and 2020, applying different nitrogen doses and observing nematode populations, disease incidence and plant growth parameters in comparison to the control. The results demonstrated that higher nitrogen levels led to a decrease in nematode populations, with reductions of 31-42% in direct-seeded rice and 23-31% in transplanted rice. Similarly, nematode reproduction factor (Rf) was lowered to less than 2, indicating reduced nematode reproduction. Root galling index (RGI) exhibited a decline, showcasing diminished root damage due to nematode infestation. Brown spot disease incidence also saw a decrease, with reductions of 2.5-2.9% in direct-seeded rice and 2.5-3.5% in transplanted rice. Furthermore, plant growth parameters like height and weight significantly improved with increased nitrogen levels, displaying the highest values at the elevated nitrogen doses. These findings underscore the importance of proper nitrogen management in controlling Rice root-knot nematode and brown spot disease in rice while fostering optimal plant growth in rice cultivation.

Key words: Brown spot disease, direct seeded rice, *Drechslera oryzae*, *Meloidogyne graminicola*, nematodes, nitrogen levels, rice, transplanted rice.

INTRODUCTION

Rice (*Oryza sativa* L.) provides food for more than half of world's population (20). Fertilizers are the main input for high-yielding rice cultivation globally. Fertilizers help in improving the soil health, yield as well as soil carbon storage (11). Besides improving the soil health, the impact of these fertilizers on soil borne pathogens needs to be studied. Throughout the growing season a number of pathogenic organisms cause different diseases. Conventionally, mineral fertilizers are applied to improve the yield and biochemical characteristics of rice. Different plant nutrients and their balanced use played a significant role in getting higher returns through enhanced yields and better quality (21). Nitrogen fertilizers increases the plant size, height, branching inflorescence as well as seed protein content (3,4,9,12). But nowadays, farmers are using fertilizers above the recommended doses without knowing their ill-effects on health and the environment. Also, the effects of these fertilizers on plant pathogens also need to be known and evaluated. Rice root-knot nematode (RRKN), *M. graminicola* and *D. oryzae* (brown spot) are two important soil-borne pathogens restricting the productivity of rice. The yield losses due to

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RRKN vary from 16-32 % (25) and 4 to 52 % losses are due to brown spot in India (27). The increasing threat of rice root-knot nematode in direct seeded rice (DSR) and the impact of brown spot disease on the quality of rice grains have raised the importance of these diseases. This study aimed to understand the effects of under and overuse of nitrogenous applications on alleviating the stress of rice root knot nematode and brown spot of rice.

MATERIALS AND METHODS

Direct Seeded Rice Experiment:

The research aimed to investigate the influence of varying nitrogen levels on the interactions between *M. graminicola* and *D. oryzae* in Direct-seeded rice. The experiment was conducted in field of Plant Breeding and Genetics during the year 2019 and 2020 in randomized block design with three replicates. The experimental plot size was 4 square meters. Three nitrogen dosage levels were considered: 112.5, 150 and 187.5 kg/ha. The experiment involved four sub-treatments: inoculation with *M. graminicola* alone at 6 days after sowing (DAS), inoculation with *D. oryzae* alone at 45 DAS, combined inoculation of *M. graminicola* (6 DAS) and *D. oryzae* (45 DAS), and a control where no nitrogen was applied. The experiment utilized rice plants of the variety PR 126. The experimental site was pre-existing with *M. graminicola* infestation. Rice seeds were directly sown in the field. Nitrogen doses were administered at 4, 6 and 9 weeks after sowing. The observations were conducted 75 days after sowing, encompassing evaluations of root-knot nematode population per 250 cc of soil, root gall index (assessed on a scale of 1 to 5), percent disease incidence of brown leaf spot, as well as growth parameters including plant height and plant weight.

Transplanted Rice Experiment:

The research aimed to assess the impact of different levels of nitrogen on *M. graminicola* and *D. oryzae* in transplanted rice. The experiment was conducted in field of Plant Breeding and Genetics during the year 2019 and 2020 in randomized block design with three replications. Three nitrogen dosage levels were examined: 78.75 kg/ha, 105 kg/ha and 131.25 kg/ha. The study included four sub-treatments: inoculation with *M. graminicola* alone at 6 days after transplanting (DAT), inoculation with *D. oryzae* alone at 45 DAT, combined inoculation of *M. graminicola* (six DAT) and *D. oryzae* (45 DAT), and a control group where no nitrogen was applied, but the pathogens were inoculated. Field pre-maintained with *M. graminicola* infestation was used for this experiment. The 30 days old nursery of rice variety named PR 114 was transplanted in the plots of size $4m^2$/treatment. Nitrogen was administered at 7, 21 and 35 days after transplanting. Inoculation with *D. oryzae* with a conidial suspension of 2×10^6 prepared and multiplied on rabbit food agar (RFA) media was done after 45 days of transplanting of PR 114. Observations were conducted 75 days after sowing and encompassed evaluations of *M. graminicola* population per 250 cc of soil, root gall index (evaluated on a scale of 1 to 5), percent disease incidence of brown leaf spot, as well as growth parameters like plant height and plant weight.

Nematode: *Meloidogyne graminicola*

Sixty days after inoculation, the data of 10-plants per plot were recorded for growth parameters [plant length (cm) and plant weight (cm)], root galling index (RGI), soil nematode population and reproduction factor. The data was pooled and then averaged over 10-replications. The plants were taken out of soil and the roots were carefully rinsed in running tap water to remove any adhering dirt particles. The root-knots were not harmed in this process. Fresh plant length and plant weight was taken immediately after uprooting the plants. For estimating *M. graminicola* population in the soil, a 250 g soil sample was taken from each plot and washed as per modified Cobb's sieving and decanting method (6). The presence of *M. graminicola* in roots was estimated based on the RGI, at 1-5 scale given by (15) Where, 1 = 0 % galls, 2 = ≤25 % galls, 3 = 26-50 % galls, 4 = 51-75 % galls, and 5 = > 75 % galls. To determine the multiplication of nematode in various treatments, the reproduction factor (Rf) was calculated as the ratio of the final nematode population to the initial nematode population

$$\text{Rf} = \frac{Pf}{Pi}$$

Where Pf: Final population; Pi: Initial population. Rf > 1: Reproduction of *M. graminicola*, while Rf < 1 : No reproduction of *M. graminicola*.

Brown spot disease: *D. oryzae*

For brown spot of rice, disease data was collected 15 and 21 days after inoculation, using the IRRI's rice Standard Evaluation System (0-9 scale) (16) where 0= no incidence; 1= less than 1 %; 2= 1-3 %; 3= 4-5 %; 4= 6-10 %; 5= 11-15 %; 6= 16-25 %; 7=26-50 %; 8= 51-75 % and 9= 75-100 % incidence. Plant length (cm) and plant weight (g) were taken 60 days after the sowing of seeds.

Statistical Analysis

Data was subjected to SPSS software. The differences among means were compared by the Tukey method at (P<0.05). The standard errors of means was calculated in Microsoft excel 2010.

RESULTS AND DISCUSSION

Effects of N levels on *M. graminicola* population in direct seeded and transplanted rice

Trials were conducted in rice root knot nematode infested soil for two years (2019 and 2020) to study the effect of different levels of N on the *M. graminicola* population. Three levels of N were taken *viz*., 112.5 kg/ha, 150 kg/ha and 187.5 kg/ha in DSR and 78.75 kg/ha, 105 kg/ha and 131.2 kg/ha in transplanted rice along with control N dose was not given. Different levels of N had a significant effect on nematode population in soil. A perusal of Table 1 revealed that root knot nematode was observed to be significantly higher where there was no addition of N in direct seeded as well as in transplanted rice while least nematode infestation was observed at higher doses of N. In direct seeded rice, there was reduction in nematode variables *viz*., soil nematode population, RGI and Rf over control with increase in N doses (Fig 1a). The control treatment with no nitrogen application showed the highest soil nematode population (466.66 nem/250cc soil) and Rf

(2.50), indicating a significant increase in nematode reproduction. However, with the application of nitrogen doses, the nematode population decreased and Rf reduced, reaching the lowest at 187.5 kg/ha (320.00 nem/250cc soil and Rf of 1.71). The RGI, which measures root galling caused by nematode infestation, decreased as nitrogen levels increased, suggesting a reduction in nematode-induced root damage. Plant height and weight also increased with nitrogen application, with the highest values observed at 187.5 kg/ha.

Table 1. Effects of N levels in direct seeded and transplanted rice on *M. graminicola* population and plant growth parameters

Nitrogen dose (kg/ha)	Soil nematode population/ 250cc soil	Rf (Reproduction Factor)	RGI (Root Galling Index)	Plant height (cm)	Plant weight (g)
Direct seeded rice					
0.0 (Control)	$466.66^{a}\pm20.81$	2.50	$3.8^{a}\pm0.2$	$50.13^{c}\pm3.90$	$30.12^{b}\pm2.18$
112.5	$376.66^{b}\pm11.37$	2.01	$3.4^{b}\pm0.1$	$63.15^{b}\pm2.39$	$37.53^{a}\pm2.24$
150	$336.66^{c}\pm3.05$	1.80	$3.2^{b}\pm0.2$	$69.23^{ab}\pm3.90$	$40.26^{a}\pm4.40$
187.5	$320.00^{c}\pm10.00$	1.71	$3.2^{b}\pm0.1$	$74.56^{a}\pm1.62$	$43.41^{a}\pm2.55$
Transplanted rice					
Control	$386.66^{a}\pm32.14$	2.07	$3.3^{a}\pm0.2$	$56.45^{c}\pm3.34$	$32.45^{c}\pm2.23$
78.75	$296.66^{b}\pm25.16$	1.58	$3.0^{ab}\pm0.1$	$67.43^{b}\pm3.30$	$40.67^{b}\pm2.51$
105	$273.33^{b}\pm5.77$	1.46	$2.8^{b}\pm0.1$	$73.34^{ab}\pm3.17$	$43.09^{ab}\pm10.40$
131.25	$266.00^{b}\pm6.02$	1.42	$2.7^{b}\pm0.2$	$79.57^{a}\pm1.87$	$46.67^{a}\pm16.28$

Initial soil nematode population- 186.66 nem/250cc soil

The values following the same letter are not significantly different according to Tukey method

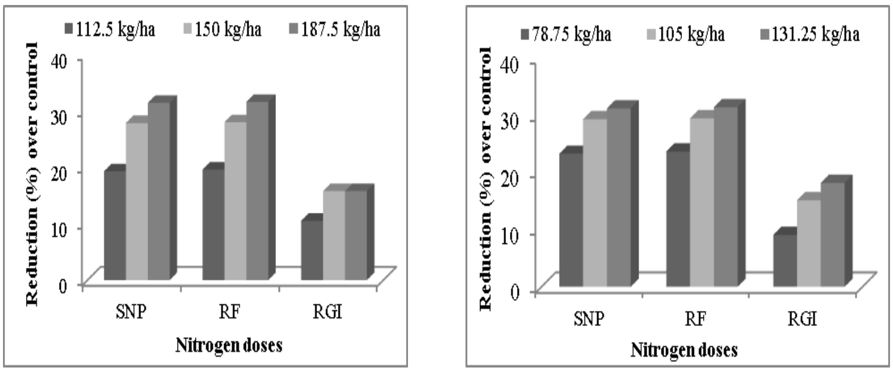

Figure 1. Reduction (%) in soil nematode population (SNP), Reproduction factor (RF) and RGI (Root Galling Index) as compared to control at different N doses in a) direct seeded and b) transplanted rice

In transplanted rice, a similar trend was observed, with the control treatment having the highest nematode population (386.66 nem/250cc soil) and Rf (2.07). As nitrogen doses increased, nematode populations decreased, and Rf values declined. The reproduction factor (Rf) of the nematode was found to be 2.07 in control whereas it was observed to be less than two at 78.75 kg/ha, 105 kg/ha and 131.25 kg/ha of N dose. Rf was observed to be decreased by 23%, 29% and 31% at 78.75 kg/ha, 105 kg/ha and 131.25 kg/ha of N dose respectively (Fig 1b). The RGI decreased with increasing nitrogen levels, indicating reduced root galling.

In the current experiment, it was found that the nematode population dropped as N levels increased. The reduction in rice root knot nematode by nitrogenous fertilizers may be due to the liberation of ammonia, which may inhibit giant cell formation and suppress nematode development (10). It might possibly be due to urea easily breaks down into ammonia by the presence of urease in the soil, which functions as a nematicide (19). Earlier Sarathchandra *et al.*, (26) reported decline in abundance of *Meloidogyne*, an obligate parasite of white clover with increasing N application. Akhtar and Mahmood (1) also reported that the addition of $(NH_4)_2So_4$ (110 kg N/ha) reduced the total population of plant parasitic nematodes as well as root galling of *M. incognita* on tomato plants. Ismail and Mohamed (18) also revealed that the application of urea at a dose of 150 unit/F significantly reduced the reproduction of *M. arenaria* infesting sugar beet under field conditions. The decrease at high levels of N may be due to accumulations of nitrate and ammonical N in soil and leads to phytotoxicity (14).

Plant height and weight was significantly increased by different levels of N in both direct seeded and transplanted rice (Fig 2a & 2b). In transplanted rice, a significant increase in plant height (79.57 cm) and weight (46.67 g) was recorded at the higher dose of N (131.25 kg/ha) as compared to control where plant height and weight was recorded to be 56.45 cm and 32.45g respectively. Growth parameters were found to be at par with each other at 105 kg/ha and 131.25 kg/ha of N dose. The same observations were recorded in DSR also. In DSR, maximum plant height was observed at the higher dose of N (74.56 cm) (187.5kg/ha of N) followed by 150 kg/ha (69.23 cm). On the other hand, minimum plant height was observed at 112.5 kg/ha of N dose. Similar trends were documented in case of plant weight. N is necessary for rice root and plant growth. Matsushima (22) also reported that a sufficient supply of nitrogen at all phases of growth supports optimal rice plant development. It also promotes the formation and maintenance of a green leaf surface throughout the plant's life cycle, allowing for optimal photosynthesis and dry matter production in the canopy. The higher plant height with N application might be attributed to the role of nitrogen in improving rice growth, internodes elongation, photosynthesis and metabolism and assimilated production (18). Olwe (24) suggests that the fertilizer (NPK) or its component(s) have a stimulatory effect on the plant leading to an enhancement of growth. N among the key nutritional elements is the most limiting nutrient for rice crop growth and productivity, requiring larger levels than other nutrients (8).

Overall, the results demonstrate that increasing nitrogen levels in both direct seeded and transplanted rice lead to reduced nematode populations and reproductive rates, as well as decreased root galling. Moreover, higher nitrogen doses positively influenced plant growth parameters, such as plant height and weight, indicating better plant growth and development. These findings highlight the importance of nitrogen management in rice cultivation for controlling *M. graminicola* infestation and promoting healthier and more productive rice plants.

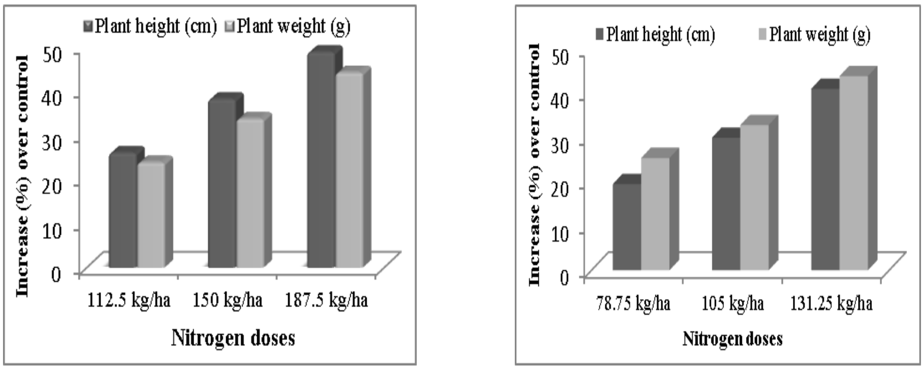

Figure 2. Increase (%) in growth parameters as compared to control in *M. graminicola* infested plants at different N doses in (a) direct seeded and (b) transplanted rice

Effects of N levels on *D. oryzae* incidence in direct seeded and transplanted rice

The effect of different nitrogen levels on the incidence of the fungal pathogen *D. oryzae*, as well as plant growth parameters in direct seeded and transplanted rice was studied. The experiment involved varying nitrogen doses, ranging from 0.0 kg/ha (control) to 187.5 kg/ha, and the results were recorded for brown leaf spot incidence, plant height, and plant weight. A perusal of Table 2 revealed that N also has an effect on brown leaf spot incidence. There was a reduction in brown spot incidence with increasing N doses (Fig 2a & 2b). In direct seeded rice, the control treatment with no nitrogen application exhibited the highest incidence of brown leaf spot (7.3 %), indicating a higher susceptibility to the fungal disease. However, as nitrogen levels increased, the incidence of brown leaf spot decreased, reaching the lowest value at 150 kg/ha (4.8 %). This suggests that higher nitrogen doses contributed to reduced disease incidence and potentially improved disease resistance in direct seeded rice. Additionally, plant height and weight increased with the application of nitrogen, with the highest values recorded at 187.5 kg/ha, indicating that increased nitrogen levels positively influenced plant growth in direct seeded rice.

Table 2. Effects of N levels in direct seeded and transplanted rice on *D. oryzae* incidence and plant growth parameters

Nitrogen dose (kg/ha)	Brown leaf spot incidence	Plant height (cm)	Plant weight (g)
Direct seeded rice			
0.0 (Control)	$7.3^{a}\pm0.2$	$52.00^{c}\pm2.00$	$35.65^{b}\pm1.76$
112.5	$6.8^{b}\pm0.2$	$57.56^{b}\pm2.41$	$38.32^{ab}\pm1.87$
150	$4.8^{c}\pm0.2$	$60.45^{b}\pm2.70$	$40.23^{ab}\pm4.03$
187.5	$4.7^{c}\pm0.2$	$67.23^{a}\pm2.54$	$43.12^{a}\pm0.85$
Transplanted rice			
Control	$7.0^{a}\pm0.2$	$57.45^{c}\pm2.7$	$37.21^{a}\pm2.3$
78.75	$6.2^{b}\pm0.2$	$63.54^{b}\pm2.2$	$40.34^{a}\pm3.1$
105	$4.3^{c}\pm0.3$	$69.23^{ab}\pm3.9$	$42.12^{a}\pm4.1$
131.25	$4.0^{c}\pm0.1$	$73.65^{a}\pm1.1$	$45.23^{a}\pm2.9$

The values following the same letter are not significantly different according to Tukey method

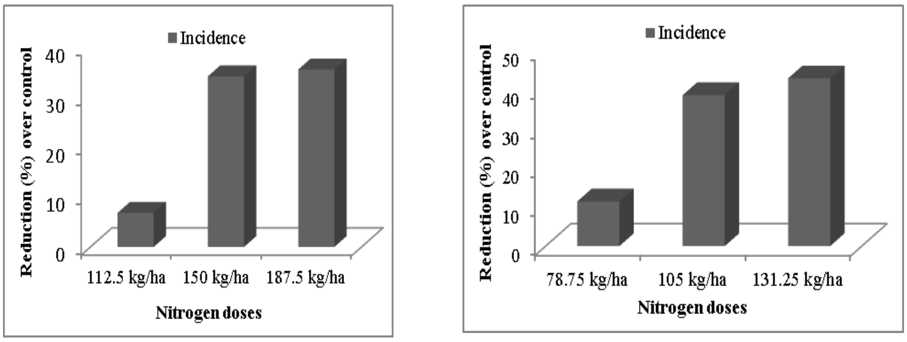

Figure 3. Reduction (%) in *D. oryzae* incidence as compared to control at different N doses in (a)direct seeded and (b) transplanted rice

Similarly, in transplanted rice, the control treatment showed the highest brown leaf spot incidence (7.0%), while higher nitrogen levels led to decreased disease incidence, with the lowest value observed at 105 kg/ha (4.3%). This indicates that nitrogen application contributed to enhanced disease resistance in transplanted rice as well. Plant height and weight also increased with increasing nitrogen doses, with the highest values recorded at 131.25 kg/ha (Fig. 4a & 4b). Overall, the results demonstrate that higher nitrogen levels in both direct seeded and transplanted rice are associated with reduced incidence of brown leaf spot caused by *D. oryzae*. The reason of reduction of disease due to nitrogen might be due to the reason that most of the infections take place through the epidermal cells instead of the stomata (23) in case of brown spot and the high N content in rice tissue may have impeded fungal penetration. It is known that N affects plant resistance by reducing the frequency of successful penetrations by some pathogens or by slowing tissue colonization upon their penetration (13). Sunder *et al.*, (30) also revealed a significant reduction in brown spot of rice with increase in N levels from 0-180 kg/ha. On

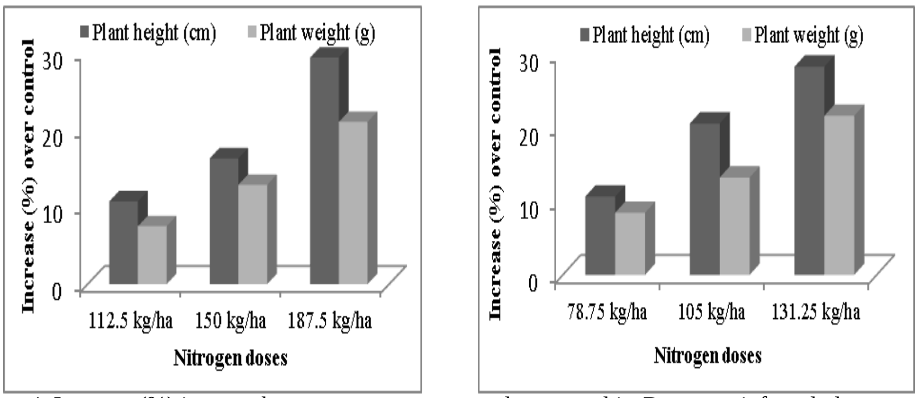

Figure 4. Increase (%) in growth parameters as compared to control in *D. oryzae* infested plants at different Ndoses in (a) direct seeded and (b) transplanted rice

mean basis, minimum brown spot incidence was recorded in the plots receiving 180 kg N/ha over other doses of nitrogen while the maximum incidence was recorded in control plots where no N was applied. Carvalho *et al.* (5) also reported that nitrogen decreased the incidence of *D. oryzae* in rice. This suggests that proper nitrogen management can contribute to improved disease resistance in rice plants. Moreover, higher nitrogen doses positively influenced plant growth parameters, such as plant height and weight, indicating better overall plant health and productivity. These findings emphasize the importance of nitrogen fertilization in rice cultivation to mitigate the impact of fungal diseases and promote healthier and more productive rice plants.

Effects of N levels on combined inoculation of *M. graminicola* population and *D. oryzae* incidence in direct seeded rice

The Table 3 presents the effects of different nitrogen levels on the combined inoculation of *M. graminicola* nematode population and *D. oryzae* incidence in direct seeded rice. The experiment involved varying nitrogen doses, ranging from 0.0 kg/ha (control) to 187.5 kg/ha, and recorded the results for soil nematode population, Rf (Reproduction Factor) for nematodes, RGI (Root Galling Index) caused by nematode infestation, brown leaf spot incidence caused by *D. oryzae*, as well as plant height and plant weight. In the control treatment with no nitrogen application, the highest soil nematode population (480.00 nem/250cc soil) and Rf (2.57) were observed, indicating a substantial increase in nematode reproduction. Moreover, the control treatment exhibited the highest RGI (4.6), suggesting severe root galling caused by nematode infestation. Additionally, the control treatment showed the highest brown leaf spot incidence (7.8%), indicating higher susceptibility to the fungal disease. However, as nitrogen levels increased, the soil nematode population, Rf, RGI, and brown leaf spot incidence all decreased. The lowest values were observed at 187.5 kg/ha, indicating that higher nitrogen doses contributed to reducing the nematode and fungal infestation in direct seeded rice. Plant height and weight also increased with nitrogen application, with the highest values recorded at 187.5 kg/ha, indicating that higher nitrogen levels positively influenced plant growth even in the presence of combined nematode and fungal stress. N affects the incidence and prevalence of various diseases and the relationship between the N fertilizers levels and plant disease incidence is complicated. In a study by Sun *et al.*, (29), it was

Table 3. Effects of N levels in direct seeded rice on combined inoculation of *M. graminicola* population and *D. oryzae* incidence

Nitrogen dose (kg/ha)	Direct seeded rice					
	Soil nematode population/ 250cc soil	Rf (Reproduction Factor)	RGI (Root Galling Index)	Brown leaf spot incidence	Plant height (cm)	Plant weight (g)
Control	$480.00^{a}\pm30.00$	2.57	$4.6^{a}\pm0.1$	$7.8^{a}\pm0.1$	$47.34^{d}\pm2.15$	$26.34^{b}\pm4.03$
112.5	$366.66^{b}\pm16.50$	1.96	$3.7^{b}\pm0.2$	$7.0^{b}\pm0.2$	$58.23^{c}\pm2.44$	$33.12^{ab}\pm3.06$
150	$320.00^{b}\pm20.00$	1.71	$3.0^{c}\pm0.2$	$6.0^{c}\pm0.1$	$62.78^{b}\pm1.25$	$36.98^{a}\pm2.75$
187.5	$326.66^{b}\pm6.02$	1.75	$3.2^{c}\pm0.2$	$5.8^{c}\pm0.2$	$68.09^{a}\pm1.71$	$39.45^{a}\pm2.24$

Initial soil nematode population- 186.66 nem/250 cc soil

The values following the same letter are not significantly different according to Tukey method found that there are many instances where N fertilizers can increase the plant incidence for example, powdery mildew, downy mildew, leaf rust and equally the opposite effects have been reported for take all, grey mould and leaf spot diseases. In addition to this, N also effects growth parameters that might be attributed due to the reason that N has the greatest impact on the growth, development, and yield of rice; it also plays a multifaceted role in maintaining and regulating the physiological functions of rice (2,7,28). N deficiency in rice hinders the synthesis of chlorophyll and proteins, thus reducing photosynthesis and affecting dry matter production (31).

Overall, the results demonstrate that higher nitrogen levels in direct seeded rice are associated with reduced soil nematode population, nematode reproduction, root galling caused by nematodes, and brown leaf spot incidence caused by *D. oryzae*. This highlights the importance of proper nitrogen management in mitigating the impact of both nematode and fungal diseases in rice cultivation. Moreover, higher nitrogen doses positively influenced plant growth parameters, such as plant height and weight, indicating improved overall plant health and productivity even under combined nematode and fungal stress.

CONCLUSIONS

The application of nitrogen exhibited dual effects on the uptake of nutrients and on the incidence and severity of two pathogens *i.e. M. graminicola and D. oryzae*. Nitrogen applied at 150 kg/ha in DSR and 105 kg/ha in transplanted rice decreased the nematode and brown spot incidence and increased the rice growth parameters. The effects of higher dose of nitrogen (150 kg/ha) in DSR was similar to 105 kg/ha dose of nitrogen in Transplanted rice. Hence, right application doses of N played crucial role in maintaining the population of these two pathogens in an eco-friendly way as well as in sustaining the growth and production of rice.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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