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GRADUATE RESEARCH ARTICLE

Maize Response to Sole and Combined Effects of Nitrogen and Nematode Stresses

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ABSTRACT

Crops grown on the field or in phytotrons are faced with different biotic stresses including plant-parasitic nematodes (PPNs) and abiotic stresses such as drought and poor soil fertility (low nitrogen levels). In this study, the interactive responses of a low-nitrogen tolerant variety LNTP-YC$_6$ and a regular variety BR-9928-DMRSR to *Pratylenchus zeae* under four nitrogen-levels: no amendment; [T0], low nitrogen [100kgN/ha NPK; T1], optimum nitrogen [200kgN/ha NPK + Urea; T2] and compost [10t/ha; T3] were investigated. The treatments were arranged in a 2 x 4 factorial fitted into randomised complete block design (RCBD) with four replicates. Data were collected on growth parameters (plant height and stem girth), yield components (number and weight of cobs), lesion score (LS), final nematode population (FNP) and reproductive factor (RF). Low nutrient stress in combination with nematode infection generally reduced maize growth and yield. Growth parameters of BR-9928-DMRSR variety were generally high while yield parameters of LNTP-YC$_6$ variety were significantly greater than in BR-9928-DMRSR variety. However, T2 and T3 improved growth and yield of both maize varieties compared to T0, with T2 being superior to T3. Meanwhile, T3 reduced FNP more than T2. FNP (107.65) and RF (1.3) of *P. zeae* on LNTP-YC$_6$ variety and with T3 was significantly low compared to T2 (178, 3.34), T0 (188, 3.6) and T1 (217, 5.0). In all the parameters considered, LNTP-YC$_6$ outperformed BR-9928-DMRSR variety. In conclusion, soil amendment with optimum rate of nitrogen and compost reduced nematode population and enhanced maize growth, while low nitrogen in combination with nematode stress reduced maize yield.

Keywords: Abiotic stress, Low-N tolerance, *Pratylenchus zeae*.

1 Introduction

Zeae mays L. commonly referred to as maize or corn, is an annual, monoic crop that belongs to the grass tribe Andropogoneae (Maydae) of the family Gramineae (Poaceae). Maize grows well in various agro-ecologies and is unparalleled to any other in its ability to adapt in diverse environments [1, 2, 3]. Globally, about 1.3 billion metric tonnes of maize is produced every year – the highest among major staple cereals [4]. In Nigeria, maize is one of the most important grains, having multiple end uses and its grain is the main economic yield product which is a rich source of starch, vitamins, proteins and minerals [3]. Its production is however constrained by biotic and abiotic influences.

Many plant pathogens and pests, including plant parasitic nematodes (PPNs) induce considerable loss during crop development and cause plant damage under moisture and other stressful conditions [5]. Plant-parasitic nematodes cause great economic losses to agricultural crops worldwide by inducing water and nutrient deficiencies [6]. Among these, *Pratylenchus* spp*.,* also known as lesion nematode (LN), are one of the most important groups of PPNs affecting crops in Sub-Sahara Africa [7]. They are migratory endoparasites that induce necrotic lesions in cereals and a range of other crops, including sugarcane, coffee, banana, legumes and potato, vegetables and fruit trees. This species of nematode is mostly associated with poor maize growth and yield [7, 8].

Similarly, nutrient imbalance is an important abiotic stress affecting plant's growth and development [9]. Lack of essential elements required for the optimum performance of the agricultural crops results in poor yield [10]. Apart from causing the physiological and morphological breakdown, nutrient imbalance or deficiency also predisposes crop to infectious diseases by breaking down plants immunity against diseases [11]. Nitrogen for example, is an essential plant nutrient required for optimum crop production. It stands out as the most important growth limiting factor especially in maize [12, 13]. Its deficiency induces chloroplast disintegration, loss of plant vigour, stunted growth and poor root development, all of which result in yield reduction [13]. According to Adejumo and Togun [14], maize grown on nitrogen deficient and degraded soil developed chlorosis, became stunted and had reduced yield.

On the field, however, plants are simultaneously faced with different environmental stresses. The stress could be biotic or abiotic and in some cases combination of both. Crop response to environmental stress therefore, varies based on the type (biotic or abiotic), severity of the stress, plant variety and combination of stresses [15]. For instance, response of a crop to single stress differs from that of combined or multiple stresses [16]. The situation of nutrient imbalance could be worsened with pest or pathogenic attack. Exposure of plants to both biotic and abiotic stresses simultaneously, induces a disruption in plant metabolism and physiology [16], and thus, leading to a reduction in fitness and productivity [17]. Many abiotic stress conditions were shown to either strengthen or weaken the defence mechanisms of plants and enhanced their resistance or susceptibility to pathogen infection [15, 18]. Available evidence indicates that simultaneous occurrence of biotic and abiotic stresses results in susceptibility or tolerance effect on plants depending on the abiotic stress and pathogen involved [19, 20].

Major stress-related research on maize have mainly concentrated on the response of the crop to a single environmental stress or a combination of two abiotic stresses without much focus on the biotic stresses especially plant-parasitic nematodes. To improve maize production in the face of different biotic and abiotic stresses, strategies must therefore be put in place to increase maize tolerance. With regards to this study, attention was given to soil fertility improvement to reduce the effect of nematode stress on maize and enhance its tolerance. Many researchers have reported the efficacy of nitrogen fertilizer and compost in reducing nematode population and improving soil fertility [21, 22]. Compost, which is an organic manure and inorganic fertilizers like Urea and NPK have been reported to improve plant nutrition and in turn induce crop tolerance to pest and diseases [14]. Similarly, the use of tolerant plant species has also been practiced to reduce plant susceptibility to environmental stress [23]. Therefore, this research investigated the effect of plant-parasitic nematodes on the growth and yield of two maize varieties (Low-Nitrogen

Tolerant Yellow Maize (LNTP-YC_6) and regular (low-nitrogen sensitive) maize variety (BR-9928-DMRSR) under nutrient stress and different nitrogen sources.

2 Materials and Methods

This study was conducted at the roof-top isolation platform of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. Two varieties of *Zeae mays*, low-nitrogen tolerant (LNTP-YC_6) and regular (BR-9928-DMRSR), were used in the study. The maize seeds were obtained from the Maize Breeding Unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2.1 Experimental procedures

Air-dried, sieved and sterilized sandy-clay soil (86.6% sand, 5.4% silt and 8.0% clay), with pH of 8.2, containing 0.166% N and 1.605% organic C, was filled into perforated 10 L (27-cm-top and 18-cm-base diameter) labelled, plastic pots. The pots were laid out in a factorial arrangement fitted into a randomized complete block design (RCBD) with the 16 treatments having three replications to make up a total observation of 48 experimental units. Two types of inorganic nitrogen fertilizers (Urea and NPK) and organic fertilizer (compost) were used as sources of the N nutrient. The inorganic fertilizers were sourced from the Department of Agronomy, University of Ibadan, Nigeria while the organic (compost) was supplied by the Crop Physiology Unit of the Department, where it was made. The inorganic fertilizers were applied at two rates to represent low N and optimum N treatments. For low N, NPK 15:15:15 was applied at 1.67 g/pot (100 kg/ha) representing 100 kg N per ha while optimum N was in two application of NPK 15:15:15 at 1.67 g/pot (100 kg/ha) plus Urea at 1.1 g per pot (100 kg/ha) delivering a total of 200 kg N/ha. The compost was applied at 25 g per pot (5 tons/ha) one week before sowing. Maize seeds were planted directly into each pot containing the soil and watered appropriately. Two weeks after emergence, the maize plants were thinned to one stand per pot.

For inorganic fertilizers, there were two treatments (NPK fertilizer alone to serve as the low N treatment and NPK + Urea to represent optimum N treatment). The pots receiving only NPK were treated at two weeks after sowing while, those receiving both NPK and urea were first treated with NPK at 2 weeks after sowing (WAS) and then, treated with urea at 8 WAS using the same rate. The quantity of NPK and Urea applied was calculated based on the percentage nitrogen present in each one of them using percentage by mass method. Percentage nitrogen in NPK and urea is 15% and 46%, respectively. Plants without nutrient application served as control.

2.2 Extraction and inoculation of *Pratylenchus zeae*

Maize roots infected with *Pratylenchus zeae* were collected from the inoculum plots of IITA, Ibadan. The inoculum of *P. zeae* was prepared by extracting live individuals from 10 g of the infected roots. The weighed roots were cut into 1-2 cm pieces and processed using the extraction tray method [24]. The extracted *P. zeae* individuals were counted from a counting dish while observing under a stereomicroscope (x16) and the number in 10 g of roots estimated. This quantity was used to determine the weight of infected root that would deliver the desired 10,000 *P. zeae* individuals per pot.

Four weeks after sowing, plants were inoculated with 10,000 individuals of *P. zeae* by incorporating 10 g of infected roots into 3-5 cm deep trenches around the roots which were afterwards covered with sterilized soil. Un-inoculated plants ($P_i = 0$) served as control. The maize plants were maintained at field capacity up to 10 weeks after sowing (WAS) after which the experiment was terminated.

2.3 Data collection

Growth parameters (plant height, stem girth, number of leaves) and chlorophyll content per plant, were collected fortnightly. Number of days to tasselling and silking per plant were also recorded. Plants were harvested at six weeks after inoculation, the roots of each plant were excised from the aerial parts, washed with tap water and biomass accumulation data were collected on fresh root and fresh/dry shoot weight. Also yield data were recorded on fresh cob weight/plant and number of cobs per plant. Severity of root lesion was determined based on a modified 1-5 rating scale for root damage [25].

Nematode extraction from the whole root system and from 250 g soil samples of inoculated and un-inoculated 10 kg pots was carried out using the extraction tray method [24]. The final nematode population was estimated by adding the total number of nematodes extracted from 10 kg pot and whole root system. The reproductive factor of the nematode (RF) was determined with the formula, RF = Pf / Pi where Pf is the final nematode count [summation of total *P. zeae* individuals from 10 kg soil and whole roots] and Pi is the initial inoculum (10,000 individuals).

2.4 Statistical procedure

Data were analysed using Analysis of Variance (ANOVA) with Generalized Linear Model (GLM) procedure of Statistical Analysis Software (SAS) System 9.2 and count data for nematode population were transformed by $\sqrt{(x+1)}$ prior to analysis. Means on growth parameters were partitioned using Least Significant Difference (LSD) at 5% probability while means on yield and nematode reproductive parameters were separated using Tukey's Studentised Range Test at 5% probability

3 Results

3.1 Growth parameters

At 8 WAS, all growth parameters of LNTP-YC$_6$ maize variety were generally higher compared to BR-9928-DMRSR variety in the presence of *P. zeae* and different amendments. Among inoculated treatments for plant height, LNTP-YC$_6$ variety amended with compost was the tallest (156.80 cm) and significantly different from the other treatments except the unamended treatment of both varieties (146.35 cm, 146.90 cm) and BR-9928-DMRSR variety amended with optimum N (145.40 cm). Among non-inoculated treatments, BR-9928-DMRSR variety amended with compost was taller (151.67 cm) and significantly different from other treatments except BR-9928-DMRSR variety amended with optimum N (139.83 cm) while LNTP-YC$_6$ variety amended with low N was the shortest plant (90.17 cm). In terms of chlorophyll content for both varieties, inoculated maize plants with amendments were significantly higher than their non-inoculated treatments at $P<0.05$. Among inoculated treatments, BR-9928-DMRSR variety amended with optimum N was highest (29.07) compared to other treatments. But worthy of note is the fact that LNTP-YC$_6$ variety amended with compost recorded a significantly higher chlorophyll content (28.45) compared to its non-inoculated counterpart (16.76). Among the inoculated treatments for the stem girth, LNTP-YC$_6$ variety amended with low N was significantly higher (1.87 cm) than other treatments except LNTP-YC$_6$ variety with no amendment (1.85 cm) as well as its non-inoculated counterpart (1.60 cm). Besides, inoculated BR-9928-DMRSR variety amended with optimum N (1.75 cm) significantly outperformed its non-inoculated counterpart (1.51 cm) which had one of the least stem girth. (Table 1). For leaf area, both inoculated LNTP-YC$_6$ and BR-9928-DMRSR with compost had significantly higher leaf area (298.13 cm^2, 314.46 cm^2) when compared to their non-inoculated counterparts (173.89 cm^2 and 271.31 cm^2) respectively. However, among the inoculated treatments, BR-9928-DMRSR variety amended with compost was significantly higher than all other treatments except LNTP-YC$_6$ variety amended with compost and low N (294.62 cm^2) respectively. Among treatments inoculated with *P. zeae* for number of leaves, LNTP-YC$_6$ variety amended with optimum N (12.50) was higher than all other treatments without much significant difference except BR-9928-DMRSR variety amended with low N (9.00) which had the lowest number of leaves. Although it was abnormally observed that the BR-9928-DMRSR variety without amendment also recorded a significantly higher number of leaves (12.50) compared to its non-inoculated counterpart. However, worthy of note is the fact that LNTP-YC$_6$ maize variety inoculated with *P. zeae* and treated with compost had a significantly higher number of leaves (12.00) compared to its non-inoculated counterpart (8.00).

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Table 1: *Interactive effects of maize varieties inoculated with Pratylenchus zeae and amended with nitrogen supplying amendments on different growth parameters and chlorophyll content*

Variety	Amendment	Plant Height (cm)		Chlorophyll content		Stem girth (cm)		Leaf Area (cm^3)		No. of leaves	
		non inoculated	*P. zeae*	non inoculated	*P. zeae*	non inoculated	*P. zeae*	non inoculated	*P. zeae*	non inoculated	*P. zeae*
BR-9928-DMRSR	No amendment	118.00	146.35*	25.73	27.70 ^{NS}	1.60	1.67 ^{NS}	268.75	255.00 ^{NS}	9.67	12.50*
	Low N	132.33	100.50*	27.23	23.70*	1.64	1.51 ^{NS}	286.67	252.64*	11.33	9.00*
	Optimum N	139.83	145.40 ^{NS}	25.87	29.07*	1.51	1.75*	241.60	249.91 ^{NS}	10.00	11.33 ^{NS}
	Compost	151.67	132.50 ^{NS}	25.60	26.60 ^{NS}	1.84	1.78 ^{NS}	271.31	314.46*	13.67	11.67*
LNTP-YC$_6$	No amendment	118.03	146.90*	30.23	28.43 ^{NS}	1.54	1.85*	231.27	255.35*	10.33	12.00*
	Low N	90.17	138.33*	24.10	25.97 ^{NS}	1.60	1.87*	238.81	294.62*	9.67	11.67*
	Optimum N	119.77	133.50 ^{NS}	24.80	28.35*	1.72	1.63 ^{NS}	242.86	226.88*	11.33	12.50 ^{NS}
	Compost	103.80	156.80*	16.76	28.45*	**1.74**	1.75	173.89	298.13*	8.00	12.00*
LSD		15.26	13.21	2.99	1.39	11.35	0.09	26.82	23.48	1.30	0.87

Data are mean values of three replications; Means with column followed by the same letter are not significantly different according to Least Significant Difference at P<0.05. *= significant, NS: non-significant indicated between nematode treatments for each parameter at P<0.05. PH- Plant height, SG- Stem girth, NOL- Number of leaves, LA- Leaf area, CC- Chlorophyll content; No inocula- no *P. zeae*, Inoculated- *P. zeae* present; Control (no amendment), Low N (15%N), Optimum N (61%N), Compost (2.26%N); LNTP-YC$_6$ (low nitrogen tolerant variety), BR-9928-DMRSR (regular variety).

It was also observed that LNTP-YC6 maize variety inoculated with *P. zeae* and treated with compost, recorded the lowest final nematode population (107.7) and reproductive factor (1.3) when compared to its other treatments and all treatments of the BB9928DMRSR variety. However, BB9928DMRSR inoculated with *P. zeae* and treated with compost recorded the least lesion score (1.0). There was significant difference observed among all treatments (Table 2).

Table 2: *Interactive effects of maize varieties inoculated with Pratylenchus zeae and amended with different nitrogen-supplying amendments on nematode population, reproductive factor and lesion score*

Maize varieties	Nitrogen Amendments	Final nematode population (FNP)	Reproductive Factor (RF)	Lesion score (LS)
LNTP-YC6	Control	187.7^{ab}	3.6^{b}	2.0^{a}
	Low N	216.5^{ab}	5.0^{ab}	2.0^{a}
	Optimum N	178.1^{ab}	3.3^{b}	1.7^{ab}
	Compost	107.7^{bcd}	1.3^{b}	2.0^{a}
BR-9928-DMRSR	Control	314.6^{a}	11.2^{a}	1.5^{ab}
	Low N	168.6^{b}	3.2^{b}	1.3^{ab}
	Optimum N	240.4^{ab}	6.1^{ab}	1.3^{ab}
	Compost	149.9^{bc}	2.3^{b}	1.0^{b}

Data are mean values of three replications; Means within column followed by the same letter are not significantly different according to Tukey's Studentized Range Test at $P<0.05$. NS: Not significant at $P<0.05$. No inocula- no *P. zeae*, Inoculated- *P. zeae* present; Control (no amendment), Low N (15%N), Optimum N (61%N), Compost (2.26%N), LNTP-YC6 (low nitrogen tolerant variety), BR-9928-DMRSR (regular variety).

3.2 Biomass production and yield parameters

Compost amendments had significant impact on biomass production compared to other treatments. BR-9928-DMRSR variety had greater fresh shoot weight while LNTP-YC6 recorded higher weight values for fresh roots. Plants under optimum N amendment had higher cob weight. BR-9928-DMRSR variety inoculated with *P. zeae* and amended with optimum N recorded the highest cob weight (104.6 g), followed by the LNTP-YC6, inoculated with *P. zeae* and amended with optimum N (104.3 g). Unexpectedly, the LNTP-YC6 variety, inoculated with *P. zeae* and amended with compost had the least cob weight (35.9 g) (Fig. 1).

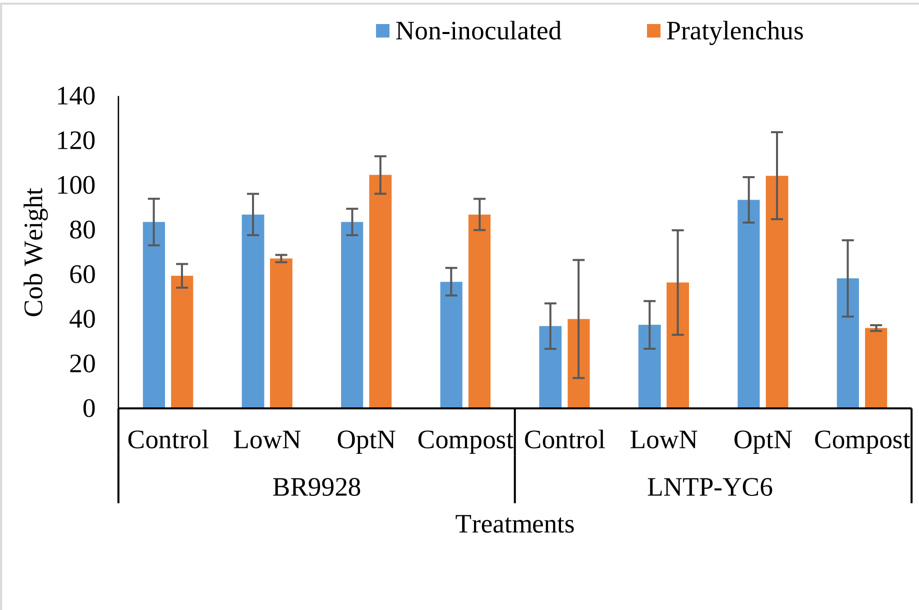

Figure 1: *Interactive effects of maize varieties inoculated with Pratylenchus zeae and amended with different nitrogen-supplying amendments on cob weight after stress*

BR-9928-DMRSR variety inoculated with *P. zeae* and amended with compost was highest in fresh shoot weight (150.5 g) compared to treatments with no amendments, both for BR-9928-DMRSR and LNTP-YC_6 varieties (Fig. 2). LNTP-YC_6 variety inoculated with *P. zeae* and amended with compost recorded the highest value for fresh root weight (84.5 g) being significantly different from the LNTP-YC_6 variety un-inoculated and amended with optimum N which had the least (32.3 g) (Fig. 3).

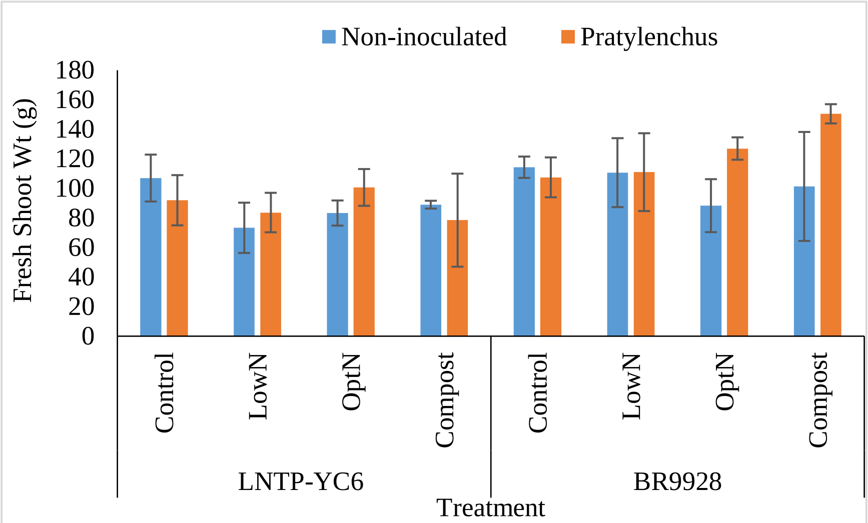

Figure 2: *Interactive effects of maize varieties inoculated with Pratylenchus zeae and amended with different nitrogen-supplying amendments on fresh shoot weight after stress*

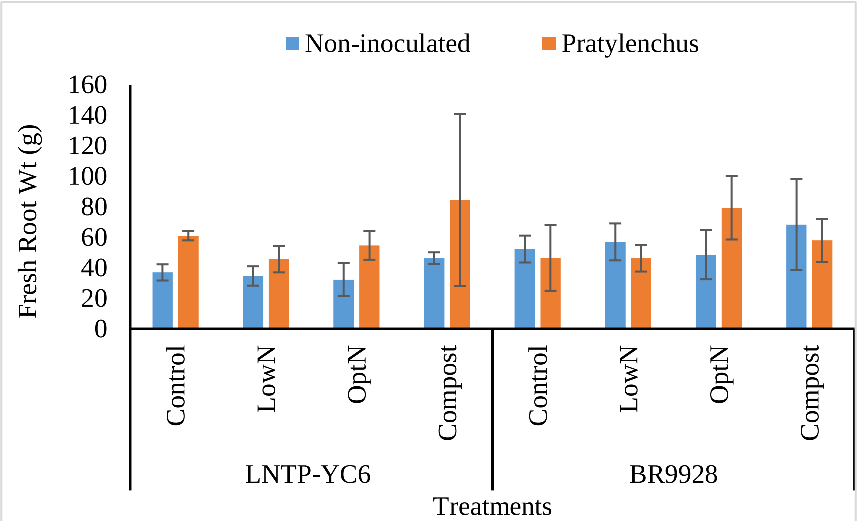

Figure 3: *Interactive effects of maize varieties inoculated with P. zeae and amended with different nitrogen-supplying amendments on fresh root weight after stress*

4 Discussion

In this study, the low N and optimum N treatments represent nutrient stress conditions (abiotic) while the inoculation with *P. zeae* represents biotic stress imposed on the maize plant. Both stresses have the ability of reducing the growth and yield of maize when imposed separately [14, 26]. However, it has been reported that crop response to single stress differs from multiple stresses. Response to combined biotic and abiotic stresses may also differ from multiple abiotic stresses. A crosstalk has been reported to occur when two or more stresses are imposed on crop simultaneously. The effects could be positive or negative, synergistic, antagonistic or additive [18] depending on the combination, plant's genetic composition and other prevailing environmental conditions.

Generally, results from this study indicate that compared to control (non-inoculated), higher nitrogen concentrations in the presence of obligate plant-parasitic nematode induces tolerance mechanism in the maize plant as observed from the growth and yield parameters. Growth and yield of maize in pots amended with optimum N showed higher yields. Tippmann et al. [19] found that simultaneous occurrence of biotic and abiotic stresses can cause either a susceptibility or tolerance effect on plants depending on the stress and pathogen under study.

Interaction of maize varieties with plant parasitic nematode at different nitrogen rates on all growth parameters showed that treatments with optimum N rate in split doses recorded higher growth and development compared to those with low N rate, no amendment (control) and compost. This indicates that the effective adoption of optimum fertilizer application in split doses can mitigate the effect of damage by *P. zeae* by improving plant metabolic and immunogenic response to parasitic activities of the microbe [27]. However, this must be carried out with adequate knowledge of the life cycle of *P. zeae*. This observation agrees with the research statement of Orisajo and Adejobi [27] that increased nutrition generally increases plant growth and nutrient accumulations in plant tissue with or without an effect on nematode population densities. Although Hu et al. [28] reported increase in disease intensity in plants as high N increased plant susceptibility to obligate pathogens, however, adequate/optimum nutrition generally increases plant growth and nutrient accumulations in plant tissue irrespective of nematode population densities. The effect of this nematode has also been attributed to alteration in the rate of water and nutrient uptake by plant roots due to the formation of root lesions [29]. This could have resulted in the reduction of the leaf chlorophyll content especially in maize inoculated with *P. zeae* and with no amendment as observed in this study. The lower chlorophyll content in the inoculated plants without fertilizer was consistent with the report of Jones et al. [29] that the presence of plant parasitic nematode influenced a reduction in chlorophyll content. The reduction in leaf chlorophyll content has however, been previously implicated in the delay of the commencement of reproductive activities due to its role in photosynthesis [29]. However, the low nitrogen tolerant maize variety (LNTP-YC_6) grown on inoculated soil amended with optimum N treatments had a better performance in the presence of *P. zeae*. This might be due to the fact that, low nitrogen tolerant variety was able to efficiently use the available nitrogen obtained from the optimum N amendment, compared to the BR-9928-DMRSR variety.

Compost treatments, in the presence of *P. zeae* inoculum, performed well in terms of biomass production. This can be attributed to the rich contribution of compost to the biomass of plants by providing nitrogen for protein biosynthesis [30]. The effects of compost amendment on the lesion score, final nematode population and reproductive factor was observed to be higher compared to the no amendment, low N and optimum amendments in the presence of *P. zeae* inoculum. It was observed that the BR-9928-DMRSR variety amended with compost had the lowest lesion score which could suggest the suppressive effect of compost on the activities of *P. zeae* on the root system. Relatively, the compost amendment reduced the transformed population of *P. zeae* individuals compared to other treatments. This is consistent with the reports of Ozores-Hampton [31] that the use of organic amendments suppressed soil phytoparasitic nematode populations by releasing some toxic chemicals to inhibit nematode microbial activity [32]. The reproductive capacity of *P. zeae* was significantly affected by the compost amendment by contributing to reduction in the juvenile population compared to other fertilizers. This suggests that compost could have the potential of being adopted as an effective means of controlling the drastic effects of obligate plant parasitic nematodes like *P. zeae*.

Though, the cob weight was observed to be the highest in treatments with optimum N compared to other treatments but this could be attributed to the positive effect of split application as suggested by [27]. Fertilizer application in split doses has also been reported to induce a kind of tolerance mechanism in the plant even in the presence of obligate parasites such as *P. zeae* [27].

5 Conclusions

From the result of the experiment, it can be concluded that improving the nutrient status of a nematode-infested soil through fertilizer application in split doses could spontaneously help increase plant's growth and development. It induces tolerance mechanism in the presence of obligate parasites such as *P. zeae*. This could be adopted as a cost-effective method of controlling the damaging effect of plant parasitic nematode on economically important arable crops such as maize. However, the use of compost, in combination with inorganic fertilizer application in sole dose, can be exploited in increasing available plant nutrients over a longer period of time as well as inducing a form of tolerance in the crop against soil-borne pathogen invasion.

6 Competing Interests

The authors declare that the research was conducted in the absence of any matter that could be interpreted as a potential conflict of interest.

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