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Efficacy of Nutrient Supplementation in Managing Groundnut Late Leaf Spot Disease Incidence through Root Feeding

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ABSTRACT: Groundnut late leaf spot disease, being an endemic foliar disease requires constant vigilance as well as frequent fungicide applications in order to sustain production. Excess fungicide usage owing to drift losses adds chemical load to environment emphasizing eco-friendly management strategy. Mineral nutrition as an effective alternative disease management strategy had wide scope but it is highly specific to plant as well as pathogen. In order to learn nutrients role in groundnut late leaf spot disease management, four nutrients K, S, Mg and Cu were selected and were studied by hydroponic sand culture using artificial inoculation with conidial suspension (@ 1 × 10^6 conidia/ ml) at 20 DAS and 35 DAS. 100 per cent Hoagland and Arnon (1950) nutrient solution was taken as base (control), twice or thrice the strength of respective nutrient over 100 per cent nutrient solution as treatments and were compared with chemical treated seeds sown in 100 per cent nutrient solution (tebuconazole 0.1%) as fungicidal check. $CuSO_4$ at thrice strength resulted in phyto-toxicity and hence deleted as treatment. $CuSO_4$ twice the strength had similar affect as fungicidal check (6.33 and 9.67 days) in delaying incubation (6.33 days) and latent periods (8.67 days) while KNO_3 thrice the strength, $MgSO_4$ thrice the strength has similar effect as fungicidal check (2.00/ leaf and 1.00 mm) with respect to lesion number (3.00/leaf) and lesion diameter (1.00 mm) resulting in lesser AUDPC values as fungicidal check compared to absolute control (4 days incubation period, 7.33 days latent period, 10.33 lesions/leaf, 2.50 mm diameter). SOD and POD enzyme activities showed similar trend corresponding to incubation period and latent period when comparing antioxidant enzyme activity of nutrient supplemented treatments with fungicidal check indicating that adequate amount of nutrient supplementation to plants offers tolerance to fungal infection through altered antioxidant enzyme activity.

Keywords: Anti oxidant enzyme, Groundnut, Late leaf spot disease, Mineral nutrition, POD, SOD.

INTRODUCTION

Arachis hypogaea, sometimes known as peanut or groundnut, is a significant oil seed crop that is a member of the legume or "bean" family. Groundnut is a valuable cash crop for both domestic and international markets in both developing and developed nations owing to its multiple uses. The average yield of groundnuts during rabi and summer is roughly 1600 kg ha^{-1}, however the output during kharif is lower, at around 1000 kg ha^{-1}. The rainfed nature of production, in addition to illnesses and insect infestations, are reasons for low productivity. Of all the groundnut diseases, late leaf spot (LLS, also known as tikka disease) caused by *Phaeoisariopsis personata* (=*Cercospora personata*) is a significant and endemic disease in the majority of groundnut growing locations. The losses estimated to be to the tune of 25.3 to 53.0 per cent in pod and haulm yields (McDonald *et al.*, 1985; Hegde *et al.*, 1995; Reddy and Rao 1999).

Providing various plant nutrients to ensure optimal plant health can enhance disease resistance through complex and multifarious mechanisms such as direct action on the pathogen, on plant growth and development, and indirectly on plant resistance (Marschner, 2012; Huber and Wilhelm 1988). Mineral nutrition can be a viable alternative disease management strategy to chemical treatment as well as disease resistance. Understanding complex interaction between disease, nutrient and environment necessitates vivid information about how a particular nutrient is actually working in crop plants to develop a strong defense system (Bhadhuri *et al.*, 2014). Dordas (2008) reported that nutrient supplements reduced disease severity when plants were under deficiency, as fertilization optimized plant growth. Among nutrients, four potential nutrients were selected based on studies on foliar diseases as follows. Mineral nutrition with Potassium improves pod yield of groundnut and aids in

tikka leaf spot disease management (Radhakrishnan, 1990; Chandrasekhar and Narayanaswami 1993). Cupric sulfate at 10^{-3} M concentration showed significant inhibitory activity (P=0.01) on *P. personata* conidia an d *Puccinia* uredospores (Kishore *et al.,* 2001). The effect of Megnesium has been in reducing the disease severity in crops like rice, wheat, citrus, potato, poppy, and peanut against foliar pathogens was also reported (Moreira *et al.,* 2015). NPK fertilizer rates significantly influence progression of foliar diseases such as leaf spot and rust in groundnut (Ihejirika *et al.,* 2006). From these studies, Potassium, Copper, Sulfur and Magnesium were selected to investigate role of nutrients in managing groundnut late leaf spot disease along with mechanism underlying in management because of nutrient specificity to plant and pathogen interaction.

MATERIALS AND METHODS

Greenhouse experiment was conducted using hydroponics technique during 2021 at Department of Plant Pathology, Agricultural College, Bapatla. Susceptible groundnut cultivar (K-6) was grown in sand culture with Hoagland and Arnon (1950) nutrient solution applied at weekly intervals. Disease- nutrient dose response was observed for four selected nutrients *i.e.,* K, Mg, S and Cu. Strength (dose) of these selected nutrients were fixed as twice or thrice the concentration used in normal Hoagland – Arnon nutrient solution. For K and Mg, two doses were selected while for Cu and S only one dose was tested as thrice the strength resulted in phytotoxicity. For each treatment three replications were maintained. Complete Hoagland and Arnon solution served as check, seeds treated with tebuconazole @ 0.1% served as fungicidal check. Conidial load in suspension was adjusted to $1x10^6$ conidia/ ml and was sprayed on to hydroponically grown groundnut plants at 20DAS and 35 DAS.

Assessment of disease: Disease development was monitored starting from first day of inoculation. The following parameters were studied for the components of resistance and data was taken from five replications.

a. The incubation period (IP), defined as days from inoculation to appearance of the first lesion, was recorded on each leaf every day from 2 to 21 days after first inoculation ad 2 to 10 days after second inoculation by scraping lesions.

b. Latent period (LP), defined as days from inoculation to the appearance of the first sporulating lesion, was recorded on each leaf every alternate day from 7 to 21 days after first inoculation ad 2 to10 days after second inoculation by scraping lesions.

c. Lesion number (LN), the average number of lesions on three quadrifoliate leaves, was counted at 10, 16 and 20 days after first inoculation, 4 and 8 days after second inoculation.

d. Lesion diameter (LD), the average diameter (in mm) of three randomly selected lesions on each quadrifoliate leaf, was measured at 12, 16 and 20 days after first inoculation, 4 and 8 days after second inoculation.

e. Area Under Disease Progression Curve (AUPDC)**:** The efficacy of different treatments will be assessed based on AUDPC values using the formula (Mc Donald *et al.,* 1985).

$$\text{AUDPC} = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i-1}) \times d$$

where,

S_i= Disease incidence at i^{th} day of evaluation

k =Number of successive evaluation of the disease

d =Interval between i and i-1 evaluation of the disease

Assessment of antioxidant enzyme activity in groundnut leaves: Fresh leaf tissue was collected from diseased and healthy plants. Approximately 200 mg of leaf tissue was weighed and ground to a fine powder in liquid nitrogen using a pre-cooled mortar and pestle. The exact weight of each powdered sample was determined before it was thoroughly homogenized in 1.2 mL of 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The samples were centrifuged at 15,000rpm for 20 min at 4ºC. The supernatant was removed, the pellet resuspended in 0.8 mL of the same buffer, and the suspension centrifuged for another 15 min at 15,000rpm. The combined supernatants were stored on ice and used to determine different antioxidant enzyme activities. Total SOD activity was assayed using a modified NBT method (Beauchamp and Fridovich 1971). For estimation of peroxidase activity is Guaiacol was used as substrate. The peroxidase activity was measured by measuring the rate of dehydrogenation of guaiacol by reacting with H_2O_2 in the presence of peroxidase enzyme. CAT activity was determined according to Aebi by measuring H_2O_2 in the reaction mixture at 240 nm (Aebi, 1984).

RESULTS AND DISCUSSION

Incubation period: After first inoculation (20 DAS), in absolute control where in 100 per cent nutrient solution was added, the incubation period for development of the disease was 10 days. In fungicidal check, *i.e.,* tebuconazole (seed treated), incubation period was 13.67 days. Among different doses of nutrient supplementation, $MgSO_4$ or KNO_3 thrice the strength resulted in longest incubation period (14 days) for first symptom expression followed by $CuSO_4$ twice the strength which was similar to fungicidal check which implies application of $CuSO_4$ twice the strength, KNO_3 or $MgSO_4$ thrice the strength has similar effect on incubation period as chemical treatment when inoculated at 20 DAS. However, KNO_3 or $MgSO_4$ twice the strength didn't show any effect on delaying incubation period and were on par with absolute control. It may also be noted that $MgSO_4$ (containing S) at twice the strength (10 days) resulted in an incubation period on par with absolute control while $CuSO_4$ (containing S) at twice the strength (13.67 days) increased incubation period significantly. This indicated that supplementation of S along with Cu is more effective in tackling the disease than with Mg. (The role of Cu as toxic metal ion to the fungus may be ruled out here as the nutrient was supplemented through root feeding). Similarly, lower incubation period in K (twice the strength) indicated that K could not sustain invading

and infecting pathogen resulting is an incubation period (10 days) on par with absolute control (Table 1).

After second inoculation (35 DAS), incubation period was least in absolute control (4.00 days) while maximum in tebuconazole seed treated plants (6.33 days). It may be seen here that incubation period was significantly lowered after second inoculation. As observed after first inoculation, treatments involving thrice the strength of KNO_3 (5.33 days) and $MgSO_4$ (6.00 days) and twice the strength of $CuSO_4$ (6.33 days) resulted in higher incubation period compared to absolute control and were on par among them. However, thrice the strength of KNO_3 was significantly lower than fungicidal check warranting probably increased dose of K. Further, it may be also interpreted that presence of N in KNO_3 might have increased plant proneness to the disease. The role of S along with Cu in decreasing the disease was further conformed here (as in after first inoculation) as $MgSO_4$ at lower dose (twice the strength) resulted in only 4 days of incubation and was on par with absolute control compared to $MgSO_4$ at thrice the strength (6 days).

Latent period: Latent period of LLS when evaluated on plants grown in different doses of nutrients, showed longest latent period in fungicidal check (22.33 days) which was on par with KNO_3 or $MgSO_4$ thrice the strength (21 days) that were in turn on par with $CuSO_4$ twice the strength (20.33 days). Plants grown in 100 per cent nutrient solution took shortest latent period (15.61 days) which was on par with KNO_3 twice the strength and $MgSO_4$ twice the strength (15.67 days) during inoculation at 20 DAS.

Second time inoculation at 35 DAS, resulted in shorter latent periods irrespective of the treatments indicating increased proneness in the plant due to prior infection. In absolute control, the latent period was shortest (7.33 days) and all the treatments were on par with absolute control except tebuconazole seed treated plants (9.67 days) and $CuSO_4$ twice the strength (8.67 days). This indicated that the effect of K and Mg was completely nullified upon second inoculation due to increased disease proneness in plants after first inoculation.

Supplementing $CuSO_4$ twice the strength had similar affect as fungicidal check in delaying latent period of LLS disease in groundnut.

Table 1: Effect of nutrient doses on incubation period and latent period of groundnut late leaf spot disease.

Sr. No.			Incubation Period days		Latent Period days	
			First inoculation *	Second inoculation**	First inoculation*	Second inoculation**
T_1	Control	100% nutrient solution	10.00 ^{c}	4.00 ^{c}	15.67 ^{c}	7.33 ^{c}
T_2	Fungicidal check	Tebuconazole 0.1%	13.67 ^{b}	6.33 ^{a}	22.33 ^{a}	9.67 ^{a}
T_3	KNO_3	twice the strength	10.00 ^{c}	3.67 ^{c}	16.33 ^{c}	8.33 ^{bc}
T_4	$MgSO_4$	twice the strength	10.00 ^{c}	4.00 ^{c}	16.33 ^{c}	8.33 ^{bc}
T_5	$CuSO_4$	twice the strength	13.67 ^{b}	6.33 ^{a}	20.33 ^{b}	8.67 ^{ab}
T_6	KNO_3	thrice the strength	14.00 ^{a}	5.33 ^{b}	21.00 ^{ab}	8.00 ^{bc}
T_7	$MgSO_4$	thrice the strength	14.00 ^{a}	6.00 ^{ab}	21.00 ^{ab}	8.33 ^{bc}
	S Em (±)		0.30	0.27	0.45	0.33
	Sed		0.43	0.38	0.64	0.47
	CD (p=0.01)		0.94	0.84	1.39	1.03
	CV %		4.32	9.25	4.11	6.89

*Groundnut plants inoculated at 20 DAS **Groundnut plants inoculated at 35 DAS

Lesion number: Groundnut plants grown hydroponically in sand culture were inoculated with *P. personata* at 20 and 35 DAS. Observations were recorded at 10, 16 and 20 days after first inoculation (Table 2).

At 10 DAI, highest lesion number was observed in control plants (1.33/leaf) which was on par with KNO_3 twice the strength (1.00/ leaf), while the rest of the nutrient supplementation treatments *viz.*, $MgSO_4$ twice the strength, $CuSO_4$ twice the strength, KNO_3 thrice the strength, $MgSO_4$ thrice the strength were similar to fungicidal check with no disease.

At 16 DAI, highest lesion number was recorded in control (1.67/leaf) and least in fungicidal check (0.67/leaf). All the treatments were on par with control in terms of number of lesions. This indicated the effect of nutrient supplementation was only to extend incubation period up to 10 DAI and to some extent extend latent period.

At 20 DAI, highest lesion number was observed in control (4.00/leaf) while the least was in fungicidal check (0.67/leaf). KNO_3 twice the strength and $MgSO_4$ twice the strength (3.00/leaf) were on par with control. $CuSO_4$ twice the strength, KNO_3 thrice the strength, $MgSO_4$ thrice the strength (1.67/leaf) had significantly lower lesion number compared to absolute control, however, higher lesion number compared to fungicidal check.

Table 2: Effect of nutrient doses on lesion number of groundnut late leaf spot disease.

Sr. No.			Inoculation at 20 DAS					Inoculation at 35 DAS					
			Lesion number 10 DAI	Lesion number 16 DAI	Lesion number 20 DAI	%Decrease over control	AUDPC	Lesion number 4 DAI	Lesion number 8 DAI	% Decrease over control	AUDPC	Total lesion number	%Decrease over control
T_1	Control	100% nutrient solution	1.33 [a] (1.52)	1.67 [a] (1.63)	4.00 [a] (2.24)		29.35	3.00 [a] (2.00)	6.33 [a] (2.71)		18.67	10.33 [a] (3.37)	
T_2	Fungicidal check	Tebuconazole 0.1%	0.00 [b] (1.00)	0.67 [b] (1.28)	0.67 [c] (1.28)	42.93	9.71	0.67 [c] (1.28)	1.33 [d] (1.52)	43.84	4.00	2.00 [c] (1.72)	49.03
T_3	KNO_3	twice the strength	1.00 [a] (1.41)	1.67 [a] (1.63)	3.00 [a] (2.00)	10.56	23.88	1.67 [b] (1.63)	3.00 [b] (2.00)	26.11	9.33	6.00 [b] (2.65)	21.39
T_4	$MgSO_4$	twice the strength	0.33 [b] (1.14)	1.00 [ab] (1.41)	3.00 [a] (1.99)	11.03	23.22	1.67 [b] (1.63)	2.33 [bc] (1.82)	32.71	8.00	5.33 [b] (2.51)	25.28
T_5	$CuSO_4$	twice the strength	0.00 [b] (1.00)	1.67 [a] (1.63)	1.67 [b] (1.63)	27.28	16.46	1.00 [bc] (1.41)	1.67 [cd] (1.63)	39.92	5.33	3.33 [c] (2.08)	38.24
T_6	KNO_3	thrice the strength	0.00 [b] (1.00)	1.00 [ab] (1.41)	1.67 [b] (1.63)	27.28	15.83	1.00 [bc] (1.41)	1.33 [d] (1.52)	43.84	4.67	3.00 [c] (1.99)	40.89
T_7	$MgSO_4$	thrice the strength	0.00 [b] (1.00)	1.00 [ab] (1.41)	1.67 [b] (1.63)	27.28	15.83	1.00 [bc] (1.41)	1.33 [d] (1.52)	43.84	4.67	3.00 [c] (1.99)	40.89
	S Em (±)		0.07 b	0.09	0.11			0.08	0.10			0.12 c	
	Sed		0.10	0.13	0.16			0.12	0.14			0.17	
	CD (p=0.01)		0.22	0.29	0.34			0.26	0.30			0.36	
	CV %		10.67	10.93	10.87			9.36	9.26			8.80	

Figures in parenthesis are square root transformed values

Fungicidal check showed 42.93 per cent reduction in lesion number followed by $CuSO_4$ twice the strength, KNO_3 thrice the strength, $MgSO_4$ thrice the strength (27.28 %). Least reduction was observed in KNO_3 twice the strength. AUDPC was least in fungicidal check (9.71) followed by $MgSO_4$ thrice the strength and KNO_3 thrice the strength (15.83) and $CuSO_4$ twice the strength (16.46%). AUDPC in plants grown in KNO_3 twice the strength (23.88) and $MgSO_4$ twice the strength (23.22) were similar to that of control (29.35). These observations at 20 DAI indicated that supplementation with selected nutrients prolonged on set of second spurt of infection as the plants inoculated.

After a gap of 15 days of first inoculation *i.e.,* at 35 DAS, groundnut plants when re-inoculated, lesion number was recorded at 4 and 8 DAI as the incubation and latent period after second inoculation were lesser than that with first inoculation. Lesion number was observed on new leaves.

At 4 DAI, in absolute control with 100 per cent nutrient supplement, lesion number was 3.00/leaf which was significantly higher compared to all other treatments. In fungicidal check, significantly least lesion number was recorded (0.67/leaf), which was on par with $CuSO_4$ twice the strength (1.00/leaf), $MgSO_4$ thrice the strength and KNO_3 thrice the strength (1.00/ leaf). Further, all the nutrient supplements had lesion number on par among themselves.

At 8 DAI, highest lesion number was obtained in control (6.33/ leaf) and least in fungicidal check (1.33/leaf). All the nutrients significantly had lesser lesion number compared to absolute control. $CuSO_4$ twice the strength treatment (1.67/leaf), $MgSO_4$ thrice the strength and KNO_3 thrice the strength (1.33/leaf) were on par with fungicidal check which also signified nutrient application $CuSO_4$ twice the strength, KNO_3 thrice the strength and $MgSO_4$ thrice the strength has similar effect in reducing lesion number as fungicidal check.

When total lesion number after two inoculations was considered, maximum lesion number was observed in control plants (10.33/leaf) followed by KNO_3 (6.00/leaf) and $MgSO_4$ (5.33/leaf) application at twice the strength while $CuSO_4$ at twice the strength (3.33/leaf) and KNO_3 and $MgSO_4$ at thrice the strength (3.00/leaf) resulted in lesser lesion number that were on par with fungicidal check (2.00/ leaf).

At the end of second inoculation, fungicidal check, KNO_3 thrice the strength, $MgSO_4$ thrice the strength and $CuSO_4$ twice the strength showed similar range of per cent reduction of lesion number over control and lesser AUDPC values compared to control followed by KNO_3 twice the strength and $MgSO_4$ twice the strength.

Lesion diameter: Lesion diameter was recorded at 12, 16 and 20 days after first inoculation done at 20DAS and at 4 and 8 days after second inoculation done at 35 DASs (Table 3).

At 12 DAI, larger lesion diameter was observed in control (0.27mm). In fungicidal check least lesion diameter was observed (0.13mm) which was on par with all the nutrient supplements except $MgSO_4$ twice the strength (0.20mm).

At 16 DAI, larger lesion diameter was observed in control (1.77mm) which was on par with KNO_3 twice the strength (1.70mm) and $CuSO_4$ twice the strength (1.70mm). Rest of the treatments *i.e.,* KNO_3 thrice the strength, $MgSO_4$ at both doses showed significantly lesser diameter compared to absolute control and were on par with fungicidal check (1.00mm). At 20 DAI, control plants recorded largest lesion diameter (4.30mm). All the treatments showed significantly lesser diameter than control. In KNO_3 and $MgSO_4$ twice the strength (3.03 and 2.90mm), lesion diameter was on par. KNO_3 and $MgSO_4$ thrice the strength (1.60mm) and $CuSO_4$ twice the strength (1.57mm) showed smaller lesion diameter compared to control and were on par among themselves. Least diameter was observed

in fungicidal check (0.43mm) which differed significantly with all other treatments.

Fungicidal check showed 48.02 per cent lesser lesion diameter while $CuSO_4$ twice the strength (30.10%) and KNO_3 and $MgSO_4$ thrice the strength showed 29.96 per cent lesser lesion diameter than control.

With second inoculation at 35 DAS, at 4 DAI observations recorded indicated that control plants had significantly largest lesion diameter (0.32mm) than that in rest of the treatments, while at 8 DAI, $CuSO_4$ twice the strength (2.00mm), KNO_3 twice the strength (1.90mm), $MgSO_4$ twice the strength (1.73mm), KNO_3 thrice the strength (1.17mm) showed significantly lesser lesion diameter than control (2.50 mm) and least lesion diameter was observed in $MgSO_4$ thrice the strength and fungicidal check (1.00 mm).

Table 3: Effect of nutrient doses on lesion diameter (mm) of groundnut late leaf spot disease.

			Inoculation at 20 DAS				Inoculation at 35 DAS		
Sr. No.			**Lesion diameter 12 DAI (mm)**	**Lesion diameter 16 DAI (mm)**	**Lesion diameter 20 DAI (mm)**	**%Decrease over control**	**Lesion diameter 4 DAI (mm)**	**Lesion diameter 8 DAI (mm)**	**%Decrease over control**
T_1	Control	100% nutrient solution	0.27 [a] (1.13)	1.77 [a] (1.66)	4.30 [a] (2.30)		0.32 [a] (1.15)	2.50 [a] (1.87)	
T_2	Fungicidal check	Tebucon azole 0.1%	0.13 [c] (1.06)	1.00 [b] (1.41)	0.43 [d] (1.20)	48.02	0.20 [b] (1.10)	1.00 [e] (1.41)	24.40
T_3	KNO_3	twice the strength	0.17 [bc] (1.08)	1.70 [a] (1.64)	3.03 [b] (2.01)	12.77	0.20 [b] (1.09)	1.90 [b] (1.70)	8.98
T_4	$MgSO_4$	twice the strength	0.20 [ab] (1.10)	1.00 [b] (1.41)	2.90 [b] (1.97)	14.22	0.17 [b] (1.08)	1.73 [c] (1.65)	11.64
T_5	$CuSO_4$	twice the strength	0.13 [c] (1.06)	1.70 [a] (1.64)	1.57 [c] (1.60)	30.41	0.20 [b] (1.10)	2.00 [b] (1.73)	7.42
T_6	KNO_3	thrice the strength	0.12 [c] (1.06)	1.00 [b] (1.41)	1.60 [c] (1.61)	29.97	0.20 [b] (1.10)	1.17 [d] (1.47)	21.32
T_7	$MgSO_4$	thrice the strength	0.13 [c] (1.06)	1.00 [b] (1.41)	1.60 [c] (1.61)	29.96	0.20 [b] (1.10)	1.00 [e] (1.41)	24.40
	S Em (±)		0.01	0.04	0.02		0.01	0.01	
	Sed		0.02	0.06	0.03		0.02	0.02	
	CD (p=0.01)		0.03	0.14	0.06		0.04	0.05	
	CV %		1.74	5.10	1.83		2.02	1.61	

Figures in parenthesis are square root transformed values

Fungicidal check resulted in 24.40 per cent lesser lesion diameter than control which was similar to KNO_3 (21.23%) and $MgSO_4$ at thrice the strength (24.40%). KNO_3 twice the strength and $MgSO_4$ twice the strength (8.98% and 11.64% respectively) had lesser lesion diameter than control. In contrast to lesion number, lesion diameter is high in case of $CuSO_4$ twice the strength.

KNO_3 thrice the strength, $MgSO_4$ thrice the strength and $CuSO_4$ twice the strength had similar effect as fungicidal check with respect to lesion number and lesion diameter.

As mobile regulator, K is essential in all cellular activities, reduction in lesion number and lesion diameter of LLS due to KNO_3 thrice the strength implied that adequate K helps to protect plant from metabolic stress and offers disease resistance. Mg increases tissue resistance to breakdown by enzymes thus involving in plant defence (Jones and Huber 2007). Sulphur offers resistance to plant diseases (Marschner, 2012) by production of toxic compounds, emission of volatile component (hydrogen sulphide, dimethyl sulphide and dimethyl disulfide), and production of gluatathione, phytoalexines and glucosinolates that were against fungal infection (Haneklaus *et al.*, 2007). Combination of Mg and S ($MgSO_4$) when administered to plants through roots at thrice the strength, reduced lesion number and lesion diameter of LLS than control and the reduction was at par with fungicidal check. As reported by Maschner (1995); Broadley *et al.* (2012) involvement of Cu in lignification of plants and cell wall stability might have increased incubation period and latent period of *P. personata* as compared to control and similar to fungicidal check when applied as $CuSO_4$ at twice the strength in our present study.

Anti-oxidant enzyme activity: Anti-oxidant enzyme activities (SOD, POD, CAT) were measured and analyzed at four days interval during disease progression which showed significant differences among treatments and pattern from 0DAI to 20 DAI.

SOD enzyme activity. Prior to inoculation, SOD activity showed non-significant difference among all the treatments (Table 4). Up to 8 DAI, $MgSO_4$ at thrice the strength showed significantly highest SOD enzyme activity compared to control, fungicidal check and nutrient supplement treatments. At 12 DAI, $MgSO_4$ at thrice the strength (0.180 units/mg fresh wt) showed highest SOD enzyme activity compared to all other treatments and was significantly on par with SOD activity of control plants (0.110 units/mg fresh wt).

Table 4: Effect of nutrient doses and *P. personata* inoculation on superoxide dismutase enzyme activity

Sr. No.			SOD enzyme activity (units/mg fresh wt)					
			0 DAI	4 DAI	8 DAI	12 DAI	16 DAI	20 DAI
T_1	Control	100% nutrient solution	0.086 [b] (1.042)	0.095 [b] (1.046)	0.155 [b] (1.072)	0.110 [ab] (1.054)	0.062 [b] (1.031)	0.095 [b] (1.046)
T_2	Fungicidal check	Tebuconazole 0.1%	0.046 [b] (1.023)	0.065 [b] (1.032)	0.085 [b] (1.042)	0.028 [bc] (1.014)	0.041 [bc] (1.020)	0.062 [b] (1.031)
T_3	KNO_3	twice the strength	0.072 [b] (1.035)	0.089 [b] (1.044)	0.102 [b] (1.050)	0.061 [bc] (1.030)	0.032 [bc] (1.016)	0.055 [b] (1.027)
T_4	$MgSO_4$	twice the strength	0.035 [b] (1.017)	0.040 [b] (1.020)	0.060 [b] (1.030)	0.014 [c] (1.007)	0.009 [c] (1.004)	0.060 [b] (1.030)
T_5	$CuSO_4$	twice the strength	0.074 [b] (1.036)	0.082 [b] (1.040)	0.102 [b] (1.050)	0.051 [bc] (1.025)	0.035 [bc] (1.017)	0.062 [b] (1.031)
T_6	KNO_3	thrice the strength	0.064 [b] (1.032)	0.084 [b] (1.041)	0.101 [b] (1.049)	0.051 [bc] (1.025)	0.024 [bc] (1.012)	0.050 [b] (1.025)
T_7	$MgSO_4$	thrice the strength	0.174 [a] (1.083)	0.280 [a] (1.131)	0.380 [a] (1.175)	0.180 [a] (1.086)	0.193 [a] (1.092)	0.217 [a] (1.102)
	S Em (±)		0.014	0.011	0.021	0.012	0.009	0.012
	Sed		0.020	0.015	0.030	0.016	0.012	0.018
	CD (p=0.01)		NS	0.033	0.065	0.036	0.027	0.038
	CV %		2.368	1.779	3.432	1.938	1.472	2.077

* Mean enzyme activities from three replications; Figures in parenthesis are square root transformed values

SOD activity of control plants was further on par with KNO_3 twice the strength (0.061 units/mg fresh wt), $CuSO_4$ twice the strength (0.051 units/mg fresh wt) and KNO_3 thrice the strength (0.051 units/mg fresh wt). Further, except $MgSO_4$ thrice the strength, all other nutrient supplements had SOD activity on par with fungicidal check (0.028 units/mg fresh wt). At 16 DAI, $MgSO_4$ thrice the strength showed highest SOD activity compared to control (0.062 units/mg fresh wt). Further, SOD activity of control was on par with fungicidal check (0.041 units/mg fresh wt), KNO_3 twice the strength (0.032 units/mg fresh wt), $CuSO_4$ twice the strength (0.035 units/mg fresh wt), KNO_3 thrice the strength (0.024 units/mg fresh wt). Significantly least activity was noticed in $MgSO_4$ twice the strength (0.009 units/mg fresh wt) which was on par with nutrient supplemental treatments and fungicidal check. At 20 DAI, $MgSO_4$ thrice the strength showed highest SOD enzyme activity (0.217 units/mg fresh wt) compared to control (0.095 units/mg fresh wt), fungicidal check (0.062 units/mg fresh wt), $CuSO_4$ twice the strength (0.062 units/mg fresh wt), $MgSO_4$ twice the strength (0.060 units/mg fresh wt), KNO_3 twice the strength (0.055 units/mg fresh wt).

There results indicated that, in control, *i.e.,* in plants supplied with 100 per cent nutrient solution and inoculated, increased SOD activity up to 8 DAI, *i.e.,* initiation of symptoms and later on decreased up to 16 DAI with a slight raise at 20 DAI indicating that SOD activities increased whenever there is fresh inoculation by the pathogen, either artificial or natural. In fungicidal check, SOD activity continued to be low compared to control indicating very low or no activity of the pathogen. All the nutrient supplement treatments also showed SOD activities lesser than the control indicating that the scavenging activity by SOD was not required to the extent seen in control plants. Which further indicates that these nutrient supplements decreased pathogen activity in host and thereby necessity of SOD scavenging activity did not arise to the extent required in untreated but inoculated host plants (control). Of all the nutrients, $MgSO_4$ twice the strength has least SOD activity indicating that $MgSO_4$ was very effective in triggering SOD activity.

POD enzyme activity: POD enzyme activity showed non-significant relationship among all the treatments during all the intervals of disease progression. (Table 5) It implies that nutrient supplementation doesn't result in significant changes in POD activity during disease progression as in case of SOD activity. In other words, in the present system of groundnut- *P. personata*-nutrient supplementation, POD activity appeared to have minimum/no role in rescuing plant cell from damage caused by reactive oxygen species produced during disease occurrence. However, it may be noted that among all the treatments, $MgSO_4$ twice the strength showed maximum POD activity especially at 16 DAI which might be due to lack of initial SOD activity. This gives a clue that when initial SOD activity is low, POD activity takes over during subsequent inoculations and vice-versa.

Table 5: Effect of nutrient doses and *P. personata* inoculation on peroxidase enzyme activity.

Sr. No.			POD enzyme activity (units/min/gram)					
			0 DAI	4 DAI	8 DAI	12 DAI	16 DAI	20 DAI
T_1	Control	100% nutrient solution	0.007 (1.003)	0.018 (1.009)	0.017 (1.008)	0.028 (1.014)	0.029 (1.014)	0.017 (1.008)
T_2	Fungicidal check	Tebuconazole 0.1%	0.031 (1.015)	0.028 (1.014)	0.007 (1.003)	0.012 (1.006)	0.013 (1.006)	0.024 (1.012)
T_3	KNO_3	twice the strength	0.025 (1.012)	0.021 (1.010)	0.009 (1.005)	0.004 (1.002)	0.023 (1.011)	0.007 (1.003)
T_4	$MgSO_4$	twice the strength	0.080 (1.039)	0.040 (1.019)	0.017 (1.008)	0.006 (1.003)	0.002 (1.001)	0.006 (1.003)
T_5	$CuSO_4$	twice the strength	0.148 (1.070)	0.049 (1.024)	0.036 (1.018)	0.012 (1.006)	0.008 (1.004)	0.003 (1.001)
T_6	KNO_3	thrice the strength	0.043 (1.021)	0.010 (1.005)	0.048 (1.024)	0.026 (1.013)	0.018 (1.009)	0.014 (1.007)
T_7	$MgSO_4$	thrice the strength	0.005 (1.002)	0.040 (1.019)	0.016 (1.008)	0.072 (1.035)	0.200 (1.094)	0.017 (1.008)
	S Em (±)		0.018	0.009	0.007	0.009	0.019	0.004
	Sed		0.025	0.012	0.010	0.012	0.027	0.005
	CD (p=0.01)		NS	NS	NS	NS	NS	NS
	CV %		2.989	1.480	1.162	1.465	3.273	0.653

* Mean enzyme activities from three replications, Figures in parenthesis are square root transformed values

CAT activity: Prior to inoculation, $CuSO_4$ twice the strength (2.154 H_2O_2/min/gram) showed significantly highest CAT activity and was on par with fungicidal check (1.731 H_2O_2/min/gram), $MgSO_4$ twice the strength (1.535 H_2O_2/min/gram) which were in turn on par with KNO_3 thrice the strength (1.152 H_2O_2/min/gram) (Table 6). $MgSO_4$ thrice the strength (0.440 H_2O_2/min/gram), showed significantly least CAT activity and was on par with control (0.195 H_2O_2/min/gram). At 4 DAI, $MgSO_4$ twice the strength (1.233 H_2O_2/min/gram) showed highest CAT activity which was on par with all the nutrient supplemental treatments except KNO_3 twice the strength 0.114 H_2O_2/min/gram) and fungicidal check (0.536 H_2O_2/min/gram). At 8 DAI, all the treatments showed non-significant relationship. At 12 DAI, $MgSO_4$ thrice the strength (1.359 H_2O_2/min/gram) showed highest CAT activity and was on par with $MgSO_4$ twice the strength (1.331 H_2O_2/min/gram), control (1.208 H_2O_2/min/gram) and KNO_3 thrice the strength (1.141 H_2O_2/min/gram). Lowest CAT activity was found in $CuSO_4$ twice the strength (0.354 H_2O_2/min/gram) which was on par with KNO_3 twice the strength (0.582 H_2O_2/min/gram) and fungicidal check (0.416 H_2O_2/min/gram). At 16 DAI, highest CAT activity was noticed in $MgSO_4$ thrice the strength (3.994 H_2O_2/min/gram) and was on par with KNO_3 twice the strength (3.962 H_2O_2/min/gram). Rest of the nutrient supplemented treatments were on par with each other, control (2.144 H_2O_2/min/gram) and fungicidal check (2.085 H_2O_2/min/gram). At 20 DAI, significantly highest CAT activity was noticed in KNO_3 thrice the strength (1.843 H_2O_2/min/gram). Further, $CuSO_4$ twice the strength (0.421 H_2O_2/min/gram) was on par with fungicidal check (0.352 H_2O_2/min/gram), KNO_3 twice the strength (0.264 H_2O_2/min/gram), $MgSO_4$ thrice the strength (0.119 H_2O_2/min/gram) and control (0.103 H_2O_2/min/gram). Significantly least CAT activity was recorded in $MgSO_4$ twice the strength (0.049 H_2O_2/min/gram) which was on par with control and fungicidal check.

Table 6: Effect of nutrient doses and *P. personata* inoculation on catalase enzyme activity.

Sr. No.			CAT enzyme activity (m mol H_2O_2 /min/gram)					
			0 DAI	4 DAI	8 DAI	12 DAI	16 DAI	20 DAI
T_1	Control	100% nutrient solution	0.195 ^{d} (1.092)	0.954 ^{ab} (1.398)	0.093 (1.045)	1.208 ^{a} (1.486)	2.144 ^{b} (1.772)	0.103 ^{bc} (1.050)
T_2	Fungicidal check	Tebuconazole 0.1%	1.731 ^{ab} (1.653)	0.536 ^{bc} (1.233)	0.120 (1.057)	0.416 ^{b} (1.190)	2.085 ^{b} (1.753)	0.352 ^{bc} (1.158)
T_3	KNO_3	twice the strength	0.546 ^{cd} (1.242)	0.114 ^{c} (1.055)	0.057 (1.028)	0.582 ^{b} (1.256)	3.962 ^{a} (2.217)	0.264 ^{bc} (1.123)
T_4	$MgSO_4$	twice the strength	1.535 ^{ab} (1.592)	1.233 ^{a} (1.491)	0.059 (1.029)	1.331 ^{a} (1.525)	1.931 ^{b} (1.710)	0.049 ^{c} (1.024)
T_5	$CuSO_4$	twice the strength	2.154 ^{a} (1.770)	1.148 ^{ab} (1.461)	0.092 (1.045)	0.354 ^{b} (1.163)	1.654 ^{b} (1.627)	0.421 ^{b} (1.191)
T_6	KNO_3	thrice the strength	1.152 ^{bc} (1.451)	1.124 ^{ab} (1.445)	0.210 (1.100)	1.141 ^{a} (1.461)	2.540 ^{b} (1.877)	1.843 ^{a} (1.682)
T_7	$MgSO_4$	thrice the strength	0.440 ^{d} (1.199)	0.565 ^{abc} (1.249)	0.147 (1.070)	1.359 ^{a} (1.535)	3.994 ^{a} (2.224)	0.119 ^{bc} (1.057)
	S Em (±)		0.082	0.084	0.021	0.038	0.107	0.053
	Sed		0.116	0.118	0.029	0.054	0.151	0.075
	CD (p=0.01)		0.252	0.258	NS	0.119	0.329	0.163
	CV %		9.922	10.874	3.423	4.852	9.811	7.747

* Mean enzyme activities from three replications; Figures in parenthesis are square root transformed values

Apparent CAT activity in control plants increased after inoculation (4 DAI) and decreased during symptom expression (8 DAI) which later increased up to sporulation (16 DAI) which again decreased at 20 DAI. KNO_3 and $MgSO_4$ at twice the strength showed similar trend as control in which incubation and latent periods were same as control. In case of fungicidal check, CAT activity decreased immediately after inoculation and increased up to symptom expression (14 DAI) and then decreased during sporulation (20 DAI). $CuSO_4$ at twice the strength, KNO_3 and $MgSO_4$ at thrice the strength also showed similar trend as fungicidal check. KNO_3 thrice the strength supplemented leaves showed increased CAT enzyme activity in diseased leaves at 20 DAI than healthy leaves during prior to inoculation while in other nutrient supplemented treatments, enzyme activity was decreased compared to healthy leaves before inoculation.

From the results, it was evident that in groundnut- *P personata*- selected nutrient supplement system; SOD and CAT enzymes played major role rather than POD. POD activity increased only when SOD activity was minimal.

SOD and CAT activities showed significant differences among nutrient supplemented treatments with *P. personata*. Nutrients irrespective of biotic stress, activates anti-oxidant enzyme activity in plant cells to offer tolerance from oxidative damage. At 1.5 mM Cu concentration, all antioxidant enzyme activities increased in leaves of the maize cultivar 31G98 while there were no significant changes in SOD and glutathione reductase (GR) activities in cultivar 3223 compared to the control except increased ascorbate peroxidase (APX) and POD activities. The lower Cu accumulation in leaves and higher antioxidant enzyme activities in cultivar 31G98 suggested an enhanced tolerance capacity of this cultivar to protect the plant from oxidative damage (Tanyolac *et al.*, 2007).

Copper treatments exhibited a non-significant change in the activities of CAT and APX, up to 10 mM Cu^{2+}, and in case of POD up to 20 mM Cu^{2+}, whereas excess Cu^{2+} in the soil led to a highly significant increase in the

activities of these enzymes in wheat during early growth stages (Azooz *et al.*, 2012).

In the present investigation, anti-oxidant enzyme activity of nutrient supplemented treatments showed changes corresponding to incubation period and latent period of LLS up on inoculation when compared to control implying that dynamics of nutrients and anti-oxidant enzymes were responsible for reduction in disease severity. SOD and POD activity showed similar trend corresponding to incubation period and latent period when comparing antioxidant enzyme activity of nutrient supplemented treatments with fungicidal check indicating that adequate amount of nutrient supplementation to plants offers tolerance to fungal infection. Similar effect of nutrients on anti-oxidant enzyme activity was reported in rice with Nitrogen and Silicon with significantly higher POD, CAT, and MDA activity at 3 and 7 days after inoculation with *Rhizoctonia solani* (Wu *et al.*, 2014).

CONCLUSIONS

Fungicidal check, KNO_3 thrice the strength, $MgSO_4$ thrice the strength and $CuSO_4$ twice the strength showed similar range of per cent reduction of late leaf spot lesion number over control and lesser AUDPC values compared to control. SOD and POD enzyme activities showed similar trend corresponding to incubation period and latent period when comparing antioxidant enzyme activity of nutrient supplemented treatments with fungicidal check indicating that adequate amount of nutrient supplementation to plants offers tolerance to fungal infection through altered antioxidant enzyme activity.

FUTURE SCOPE

The effective nutrient combinations identified in this study can be tested for other foliar pathogens and can be applied for commercial use to reduce chemical use and increase production.

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Conflict of Interest. None.

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