OPEN ACCESS Int. J. Phytopathol. 07 (01) 2018. 19-29 DOI: 10.33687/phytopath.007.01.2393

Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

INFLUENCE OF DIFFERENT NITROGEN SOURCES ON GROWTH AND PATHOGENIC CAPABILITY OF *RHIZOCTONIA SOLANI* CAUSING ROOT ROT OF FABA BEAN

Maha H. Mohamed, Mostafa H. Mostafa *
Plant Pathology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

**Corresponding Author Email: mostafa_mohamed@agr.asu.edu.eg*

A B S T R A C T

The effect of different nitrogen sources (glucosamine sulfate, ammonium sulfate, aspartic acid, phenylalanine and peptone) in comparison to sodium nitrate, the major nitrogen compound in basal agar Czapek's synthetic medium growth were studied on the linear growth of *Rhizoctonia solani* and its pathogenicity on faba bean germinated seeds. Ammonium sulfate exhibited faster liner growth and showed the same effect as the basal medium with sodium nitrate while glucosamine sulfate showed less growth rate compared with sodium nitrate. Glucosamine sulfate and ammonium sulfate showed a significant reduction in a number of infection cushions which led to significant decrease of disease index *in vitro*. Under greenhouse conditions, glucosamine sulfate or peptone as a sole nitrogen source in food requirements of *Rhizoctonia solani* inoculum depressed the virulence of the fungus. The effect of different amounts of glucosamine sulfate was determined on fungal growth rate, infection cushions, disease index *in vitro* and polyphenol oxidase activity. Increasing amount of glucosamine sulfate showed significant reduction of growth rate in comparison to the basal medium with sodium nitrate. All seeds subjected to *R. solani* grown on different amount of glucosamine sulfate showed the lower number of infection cushions, disease index and polyphenol oxidase activity compared with sodium nitrate. Under greenhouse conditions, disease index showed a significantly decreased effect when glucosamine sulfate used as soil applications and showed the better effect on shoot weight and root weight compared with control plants treated with sodium nitrate. Our study proposes that glucosamine sulfate may act as controlling factor of pathogenicity genes of *R. solani*.

Keywords: *Rhizoctonia solani*, faba bean, *Vicia faba*, nitrogen sources, glucosamine sulfate, pathogenicity.

INTRODUCTION

Nitrogen is an essential element for the growth of all living organisms including fungi and soil microorganisms. Fungi possess the ability to use organic or inorganic substances of nitrogen as food requirement and its development (Marzluf, 1997). The form of nitrogen that available to plant pathogenic fungi effect on all the cell activities, such as growth, sporulation, or pathogenicity genes. The ability of fungi to use various forms of nitrogen and its effect on the hyphal morphology, growth and sporulation has been investigated. For instance, (Ritter, 1909; Bach, 1927) showed that *Rhizopus nigricans* grew well in mineral nutrient solutions which contained ammonium sulfate or ammonium nitrate but not in those which contained sodium nitrate. The authors explained the result to the inability of *Rhizopus nigricans* to assimilate nitrate as nitrogen. The colony and hyphal morphology of *Verticillium albo-atrum* were affected and a reduction of the radial growth rate when *V. albo-atrum* grown on medium containing glucosamine (White and Gadd, 1983). Eight different nitrogen sources (ammonium nitrate, asparagine, glutamine, glycine, potassium nitrate, sodium nitrate, sodium nitrite and tryptophan) were tested on the mycelial dry weight of eight *Fusarium* spp. isolated from agriculture soil in West Bengal, India. Organic nitrogen sources (except glutamine) were found more effective to increase the mycelial growth of all tested isolates than the inorganic nitrogen sources. On the otherwise, sodium nitrate was exhibit the best one of inorganic nitrogen compounds to increase the growth of all *Fusarium* tested isolates (Islam, 2015).

As well, among of different nitrogen sources including KNO_3, $NaNO_3$, peptone, tryptone, ammonium nitrate, and urea Jabin and Nasreen (2016) evidenced that KNO_3 were found

to be the favorite source of nitrogen for growth of *Alternaria solani* followed by $NaNO_3$, peptone and tryptone, they stimulated increasing the growth while urea supported the poorest growth. On the other side, little of researchers reported that the nitrogen source proposed to act as a regulatory switch to stimulate expression of pathogenicity related genes in plant pathogenic fungi. For instance, ammonia salts stimulated diseases caused by *Rhizoctonia, Fusarium* and *Sclerotium* on tomato, cotton, sugar beet, wheat and citrus. On the contrary, the form of nitrate is favoured to another pathogen such as *Pythium* causing root rots in corn and pea (Huber and Watson, 1974).

Mycelial growth of *Phytophthora cinnamomi* the causal of root rot of avocado was significantly increased by amended broth media with nitrate nitrogen, while no significant effect was observed when media supplemented with urea or ammonium nitrogen compared with unamended broth media. Avocado seedling treated with ammonium sulfate showed significantly decreased of disease severity of the fungus than when nitrate nitrogen or urea was used (Duvenhage *et al.*, 1992). The aim of this study was to evaluate the effect of different nitrogen sources on growth and their amendment in inoculum substrate on the pathogenic capability of *Rhizoctonia solani* growth.

MATERIALS AND METHODS

Rhizoctonia solani AG4-HGI (Maha Helmy, 2015) and faba bean seeds cv. Giza were used throughout this study.

Effect of different nitrogen sources on linear growth and pathogenicity *in vitro*

On growth: The effect of nitrogen sources on the linear growth of *Rhizoctonia solani* was carried out by growing a piece of young hyphae on Petri dishes (9 cm) containing the basal agar Czapek's synthetic medium consisting of 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$. $7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, 1 ml of $ZnSO_4$, $CuSO_4$, 30 g sucrose, 20 g agar and distilled water up to 1 litre. Sodium nitrate of the basal medium was substituted with equivalent units of nitrogen compounds of glucosamine sulfate, ammonium sulfate, aspartic acid, phenylalanine, and amount of peptone added was equal to the weight of sodium nitrate that presents in the basal Czapek's Dox agar medium. Before sterilization, the pH of all media was adjusted to 7.0 with 0.1 NaOH or HCl. The diameter of growth rate was recorded after 72 hr. The growth rate was measured with six replicates.

Pathogenic capabilities: To determine the effect of different nitrogen sources on the virulence of *R. solani,* sterilized faba bean germinated seeds were putting on fungal growth plates growing on each nitrogen sources, 18 hours after that five seeds were randomly taken for the account of infection cushion as described below. Plates were incubated at 25 ± 1˚C for three days in the dark, and then disease index was determined according to (Mohamed *et al.*, 2014). Six dishes (each contains 5 seeds) were used as replicates.

Microscopic examination: Eighteen hours after germinated faba bean seeds had been put on fungal growth, the lower epidermal surface (the site of infection) of the hypocotyls was stripped and stained with 0.1% trypan blue in 5% lactophenol then transferred to a glass slide for examined with a light microscope (Leica DM 2500) in Histopathology Unit, Dep. Plant Pathol., Fac. Agric., Ain Shams University. Five seeds of each treatment were taken for the account of infection cushions in 5 microscopic fields/seed (25 microscopic fields/treatment).

Effect of preparing the fungal inoculum on different nitrogen sources on its pathogenicity

Preparation of inoculum: To evaluate the effect of different nitrogen sources on the pathogenicity of *R. solani* under greenhouse conditions, sodium nitrate of the basal Czapek's Dox broth medium was replaced with equivalent amount of nitrogen concentration of different nitrogen sources previously mentioned and equal amount of peptone.

Dried sand washed with hydrochloric acid and sterilized in the autoclave was placed in 9 cm Petri dishes (120 g sand/plate). Each different nitrogen sources in Czapek's Dox broth media was added separately to the sand dishes (25ml/plate). Dishes were infested by actively *R. solani* growth. Plats were incubated at 25 ± 1˚C in the dark for 10 days. Dishes with fungal growth were used for an infestation of sandy pots.

Infestation of sandy pots and cultivation of seeds: Washed and sterilized sand was distributed in plastic pots (10 cm diameter), each pot contains approximately 430 g of sand. Pots were infested with fungal inoculum growing on different nitrogen sources (one plate/pot). Infested pots were left for six days with follow up irrigation. Sterilized germinated faba bean seeds were sown in sand pots infested with *R. solani*. Pots were irrigated when needed with sterilized tap water.

Determination of disease index and plant growth characters: Twenty days after planting, number of plants emergence was calculated. Plants were taken and washed to release adhered sand for determination of plant growth

parameters as plant high (cm), number of leaf per plant, foliage weight (g), and root weight (g). These parameters were determined 28 days after sowing. Disease index was determined according to (Maha Helmy, 2015).

Effect of different amounts of glucosamine sulfate on linear growth and disease index *in vitro:* In another experiment, the effect of different amounts of glucosamine sulfate on fungal growth and on disease index *in vitro* was studied. The effect of each amount on the linear growth and on the virulence of *Rhizoctonia solani* were achieved by growing young hyphae on Petri dishes (9 cm) containing the basal agar Czapek's synthetic medium. Sodium nitrate of the basal medium was substituted with half, equivalent and double units of glucosamine sulfate (5.89, 11.78 and 23.56 g/L, respectively) and used the basal medium with optimum quantity of sodium nitrate (3g/L). The pH of all media was adjusted to 7.0 before sterilization. Diameter of growth rate (after 72 hr) and disease index (3 days after putting sterilized germinated faba bean seeds on the fungal growth) were determined as described above.

Determination of polyphenol oxidase activity: Seeds of each treatment were taken 72 hours after inoculation and grinded in phosphate buffer solution (1:2, w:v – pH 6.0). Suspensions were centrifuged at 10000 rpm for 10 min at 4°C. The supernatant was taken for PPO activity and determination was carried out by using catechol as substrate. The reaction mixture contained freshly prepared of 0.25 ml of crude enzyme, 0.25 ml of 50 mM catechol and 1 ml of 0.1 M phosphate buffer, pH 7.0. Enzymatic activity was determined by measuring the increase of optical density at 410 nm (Ünal, 2007). The absorbance was recorded after 15 minutes using an ultraviolet-visible spectrophotometer, (Unico UV-2100) USA. All determinations were determined in triplicate.

Effect of different amount of glucosamine sulfate on disease index and plant growth characters under greenhouse conditions

Preparation of inoculum and infestation of sandy pots: Sand washed with hydrochloric acid and sterilized in the autoclave was placed in 9 cm Petri dishes (120 g sand/plate). Czapek's Dox broth media was added to the sand dishes (25ml/plate). Dishes were infested by actively *R. solani* growth. Plats were incubated at 25 ± 1˚C in dark for 10 days. Dishes with fungal growth used for an infestation of sandy pots.

Washed and sterilized sand was distributed in plastic pots (10 cm diameter), each pot contains approximately 430 g of sand. Pots were infested with the fungal inoculum (one plate/pot). Infested pots were left for six days with follow up irrigation.

Cultivation of seeds and determination of plant growth characters: Germinated faba bean sterilized seeds were sown in sand pots infested with *R. solani* or not. Each pot was received 30ml of different amount of glucosamine sulfate (5.89, 11.78, 23.56 g/L, respectively), and sodium nitrate (3g/L) or distilled water as a control. Each treatment included infested and non-infested pots (9 pots/ application). Pots were irrigated daily with tap distilled water. After 20 days from planting a number of emerging plants was calculated and plant growth characters were determined 28 days after sowing as above.

Statistical analysis: Experimental data were statistically analyzed by analysis of variance (ANOVA) using The Statistical Analysis System (SAS) (Littell *et al.*, 1996). Standard deviation (SD) was calculated according to (Ghahramani, 2000).

RESULTS

Five nitrogen sources: glucosamine sulfate, ammonium sulfate, aspartic acid, phenylalanine and peptone were replaced with the equivalent of nitrogen units in the basal agar Czapek's synthetic medium as the sole nitrogen source to determine their effect on growth rate and pathogenicity of *Rhizoctonia solani* as well as the basal constituent of Czapex's medium.

The effect of growing *R. solani* in Czapek's medium amended with different nitrogen compounds on fungal growth and its pathogenicity on germinated faba bean seeds: Results of growth rate that represent in figure (1) indicate that growth of fungus on medium with ammonium sulfate is the better source of nitrogen that showed faster growth and showed equal effect with the medium amended with sodium nitrate on growth. Meanwhile, other nitrogen sources led to a downward trend of growth rate, and the medium with glucosamine sulfate showed less growth rate of *R. solani* compared with the basal medium. The effect of nitrogen sources on pathogenicity were determined *in vitro*, glucosamine sulfate and ammonium sulfate showed significant reduction of disease index on faba bean germinated seeds put on mycelial hyphal growth (2.3 and 2.6, respectively) compared with sodium nitrate (5.0). On the otherwise, aspartic acid, phenylalanine and peptone showed the same effect on disease index as $NaNO_3$ (Figure 2).

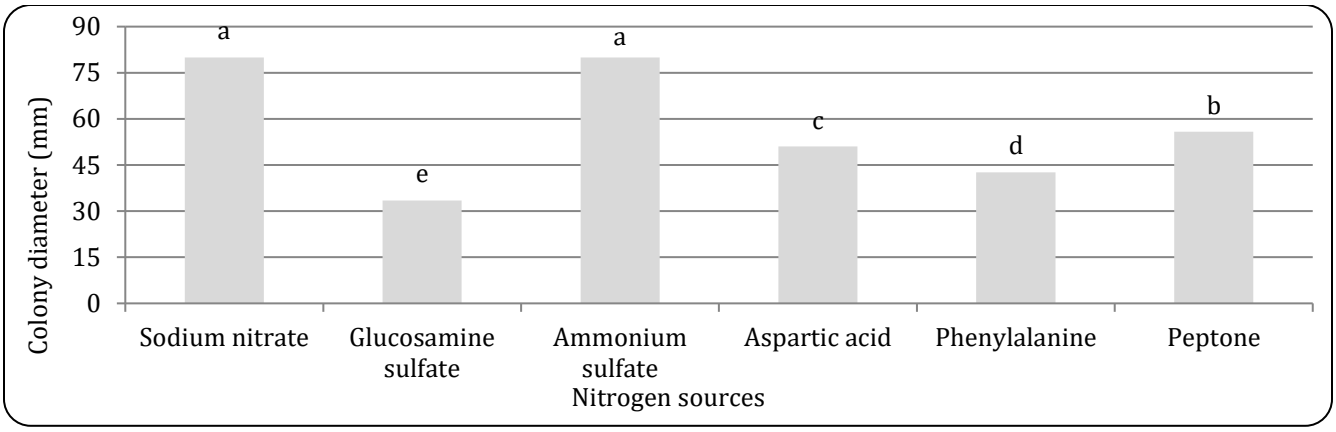

Figure 1. Effect of different nitrogen sources on colony radial growth of *Rhizoctonia solani in vitro* 72 hours after sub-culturing.

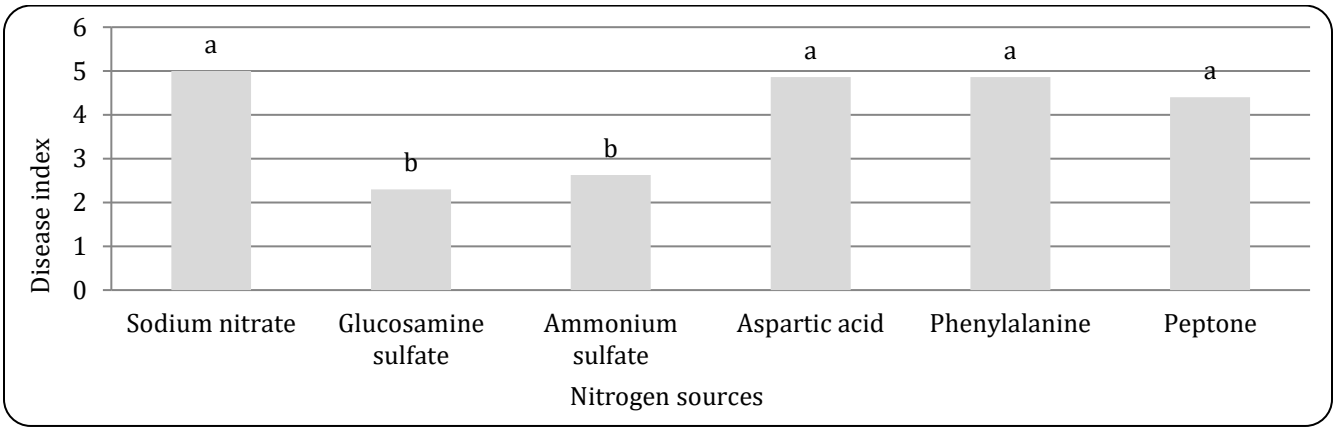

Figure 2. Effect of different nitrogen sources on disease index of *R. solani* on faba seeds *in vitro*.

Number of infection cushions for all treatments were accounted 18 hours after hypocotyls had been subjected to the fungal growth, significant decrease of infection cushions were formed when glucosamine sulfate, ammonium sulfate and peptone were used as sole source of nitrogen. According to statistical analysis it distinguished four categories in descending order concerning No. of infection cushions: the first include only sodium nitrate, the second include aspartic acid and phenylalanine, the third include ammonium sulfate and peptone, and the last category include glucosamine sulfate (Figure 3).

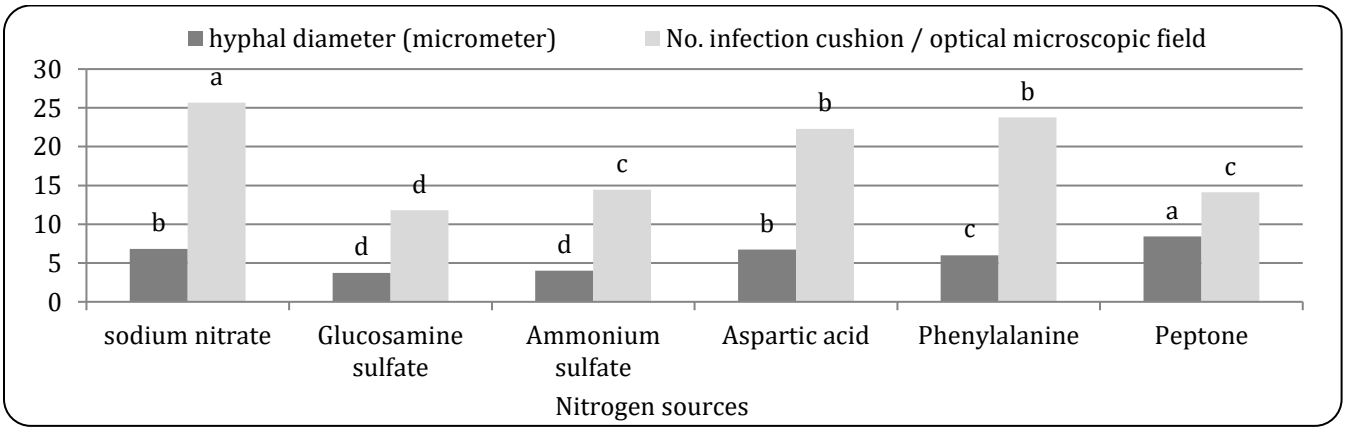

Figure 3. Effect of different nitrogen sources on mean number of infection cushions and hyphal diameter of *R. solani* formed on lower epidermal surface of faba bean hypocotyls 18 hours after inoculation.

The hyphal diameter of infection cushions were recorded for all treatments, glucosamine sulfate or ammonium sulfate led to significant reduction of hyphal diameter compared with sodium nitrate (Figure 3 and 4). Results of this study showed strongly significant positive correlations between disease index and both of diameters of hyphae and number of infection cushions for all treatments (+ 0.82 and + 0.84, respectively). The very weak correlation coefficient between disease index and linear growth of *R. solani* is recorded (+ 0.11).

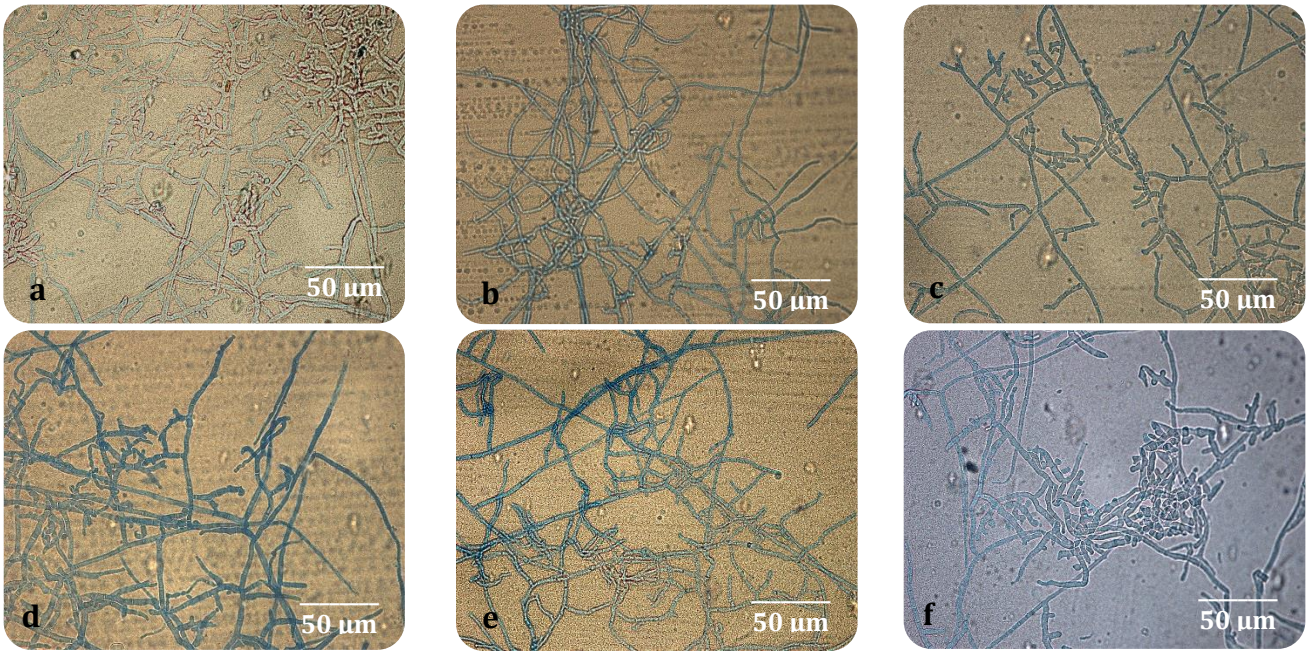

Figure 4. Effect of different nitrogen sources in Czapek's Dox agar medium on infection cushions and hyphal diameter of *R. solani* produced after 18 hr. on lower epidermal surface of faba bean hypocotyls. a: sodium nitrate, b: glucosamine sulfate, c: ammonium sulfate, d: aspartic acid, e: phenylalanine, f: peptone.

Effect of preparation of *R. solani* inoculum on different nitrogen sources on its pathogenicity under pots experiment: Sand amended with Czapek's medium in which nitrogen source was replaced by different nitrogen sources was used for the preparation of the fungal inoculum. These inocula were used for an infestation of pots containing sand for seeding faba bean seeds. According to the obtained results, different inocula showed different effects on disease index on faba bean root. Inocula prepared on sodium nitrate, ammonium sulfate or phenylalanine gave the higher disease index followed by aspartic acid and peptone, respectively. Inoculum prepared on glucosamine sulfate led to the lower disease index (Figure 5).

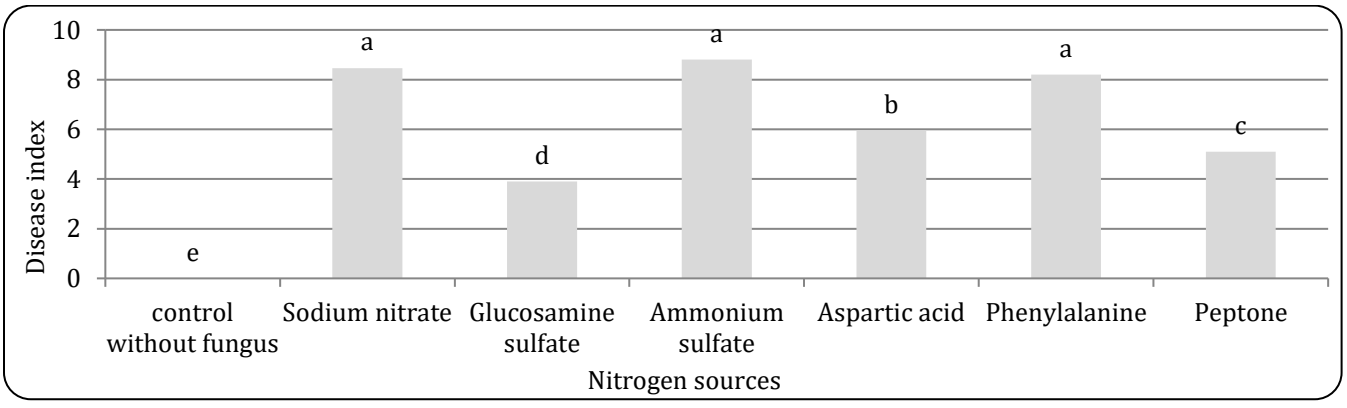

Figure 5. Effect of inoculum growth on different nitrogen sources on disease index of *R. solani* on faba bean plants under greenhouse condition.

Glucosamine sulfate showed better one on seedling emergence and showed significantly improvement of all plant growth characters (Figure 6). Morphological features of root system were varied from inoculum to another. Ammonium sulfate, aspartic acid, sodium nitrate and phenylalanine showed very weak root in comparison to root growing in non-infested soil or that inoculated by fungal inoculum prepared on glucosamine sulfate (Figure 7).

Figure 6. Effect of inoculum growth of *R. solani* on different nitrogen sources on plant growth characters of faba bean plants. 1: sodium nitrate, 2: aspartic acid, 3: phenylalanine, 4: ammonium sulfate, 5: peptone, 6: glucosamine sulfate, 7: control without fungus. (Photographs illustrating; a: percentage of plant emergence, b: stem length).

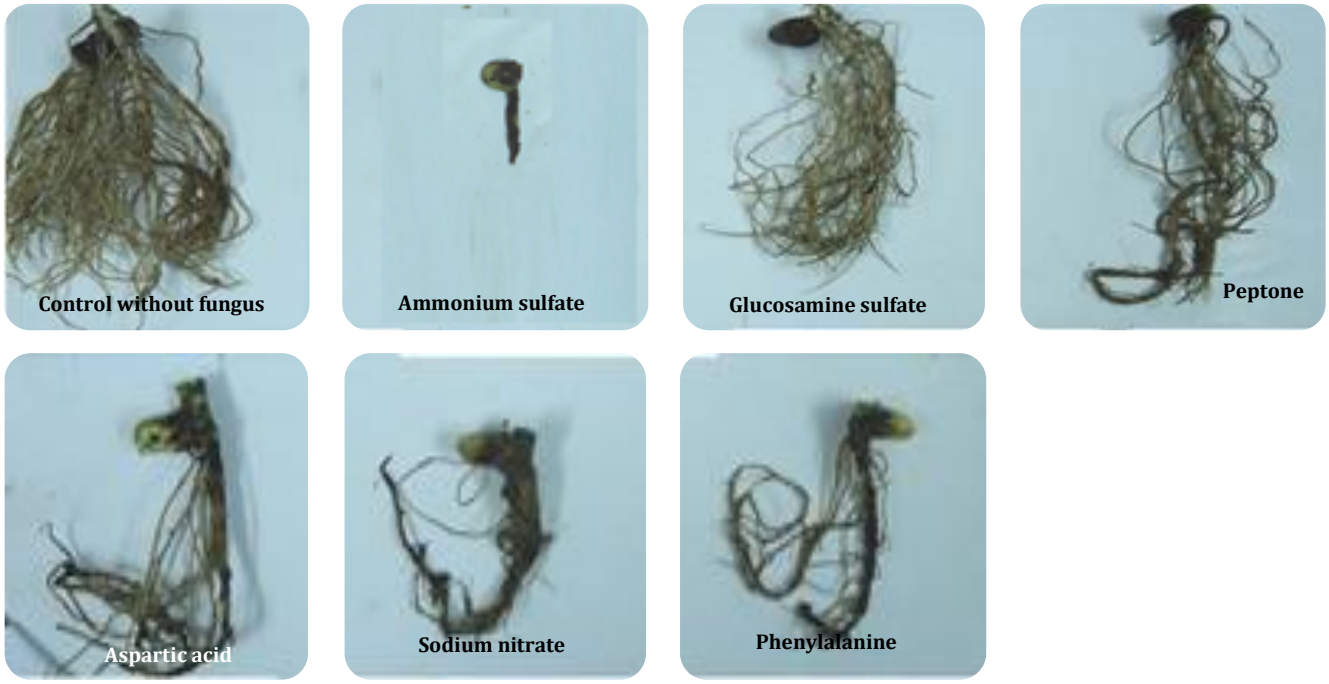

Figure 7. Effect of inoculum growth of *R. solani* on different nitrogen sources on root morphology of faba bean plants.

Effect of growing *R. solani* in Czapek's medium amended with different amount of glucosamine sulfate on fungal growth, morphology and its pathogenicity on germinated faba bean seeds: The effect different amount of glucosamine sulfate was determined on fungal growth rate and disease index. The results of the growth rate of *R. solani* indicated that increasing amount of glucosamine sulfate in Czapek's agar medium showed significant reduction of growth rate compared with sodium nitrate (Figure 8). It has been observed that no sclerotia were formed on all concentrations of glucosamine sulfate compared with sodium nitrate in the basal agar Czapek's synthetic medium. In the laboratory, all different amounts of glucosamine sulfate showed significantly depressed of disease index compared with sodium nitrate (Figure 9). Significantly fewer number of infection cushions were produced on the lower surface of hypocotyls for all amounts of glucosamine sulfate (Figure 10).

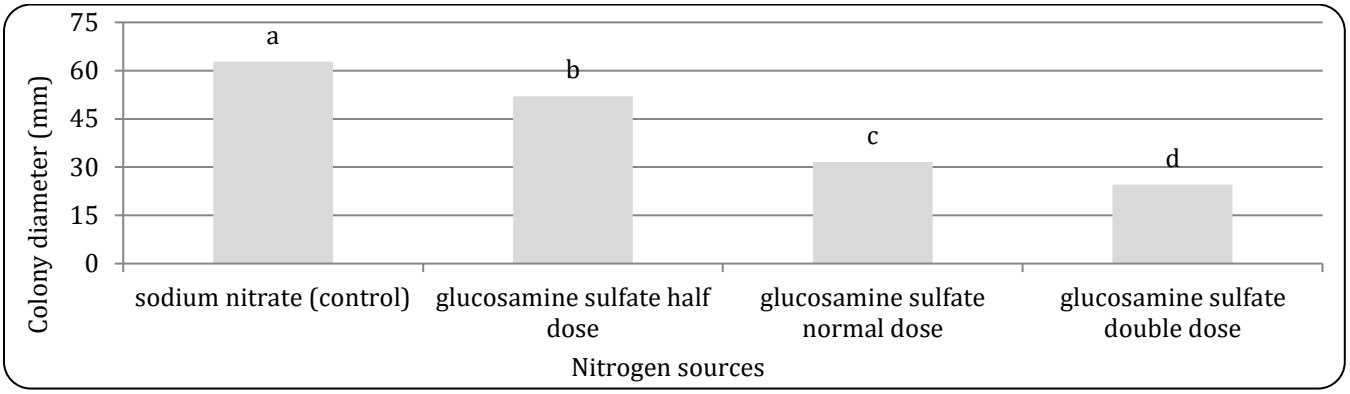

Figure 8. Effect of different amount of glucosamine sulfate on radial colony growth of *Rhizoctonia solani in vitro* 72 hours after sub-culturing.

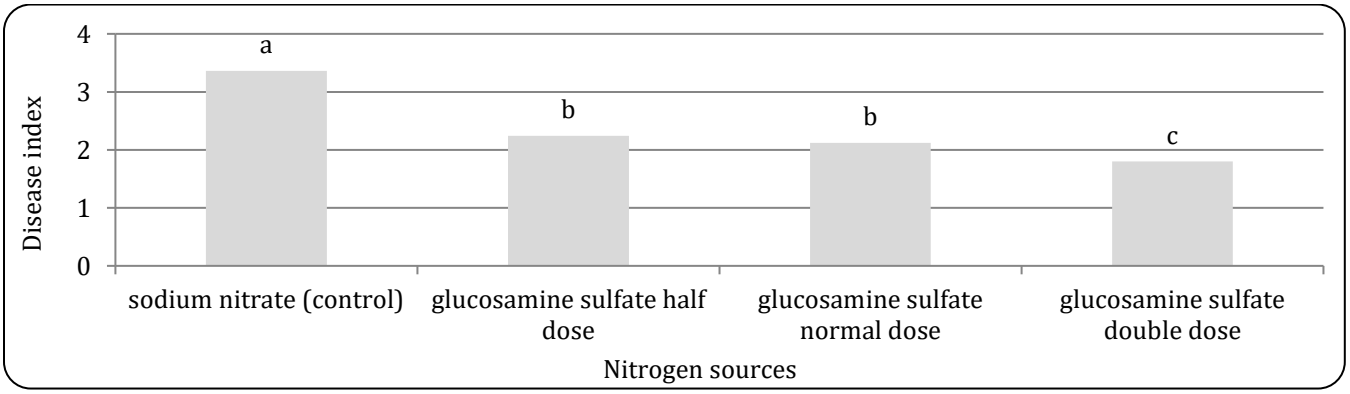

Figure 9. Effect of different amount of glucosamine sulfate on disease index of *Rhizoctonia solani in vitro.*

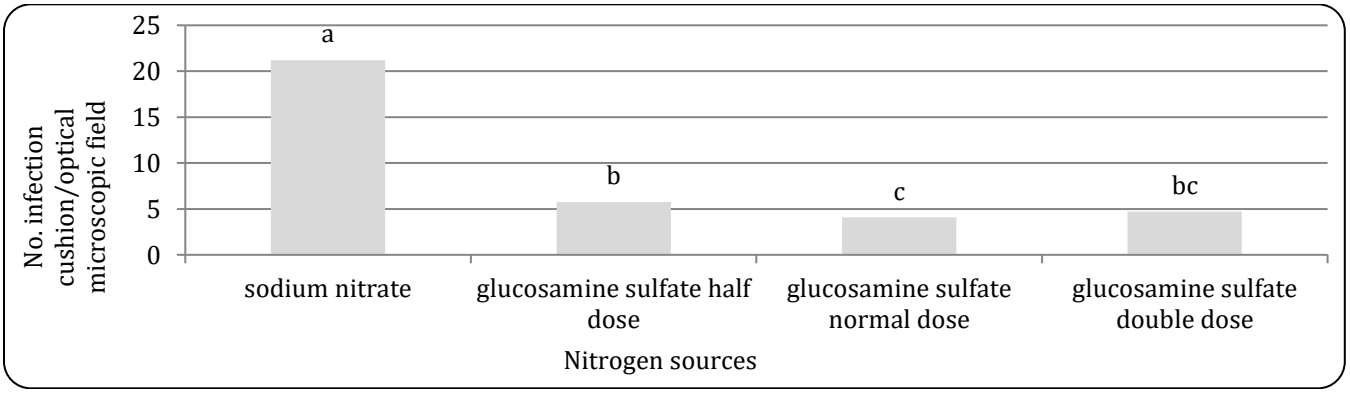

Figure 10. Effect of different amount of glucosamine on mean number of infection cushions of *R. solani* produced on faba bean seeds 18 hours after inoculation.

Polyphenol oxidase activity in faba bean germinated seeds subjected to *R. solani* growth on different amount of glucosamine sulfate showed significantly decreased in enzymatic activity compared with sodium nitrate (Figure 11). Under greenhouse conditions, all amounts of glucosamine sulfate significantly decreased disease index (Figure 12). On plant growth characters, no significantly effect on both stem length and number of leaves/plant of all treatments. On the otherwise, all different amount of glucosamine sulfate showed significantly increased effect on shoot weight and root weight compared with control plants treated with sodium nitrate (Table 1 and Table 2).

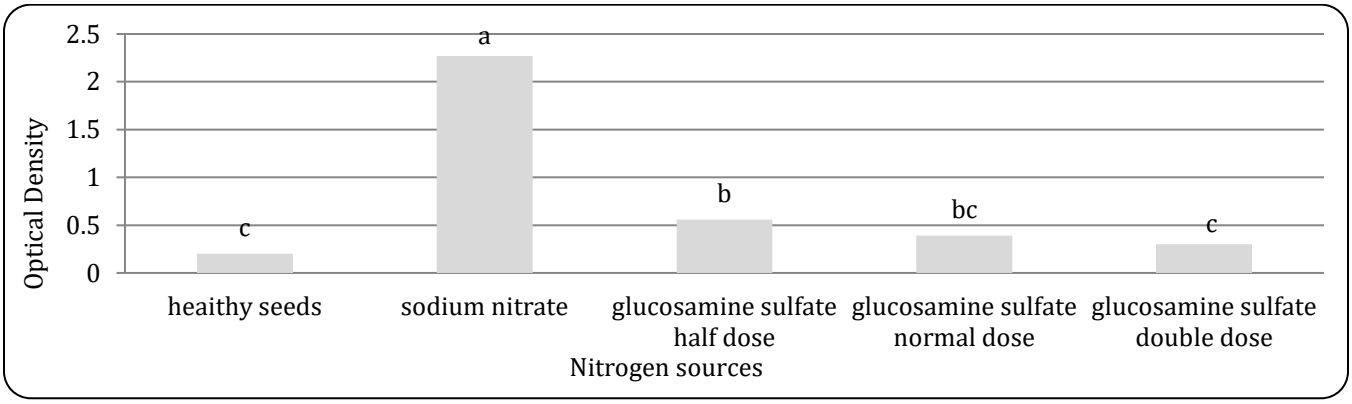

Figure 11. Polyphenol oxidase activity in faba bean seeds subjected to *R. solani* growth on different amount of glucosamine sulfate as a sole source for nitrogen. The absorbance was recorded at after 15 minutes.

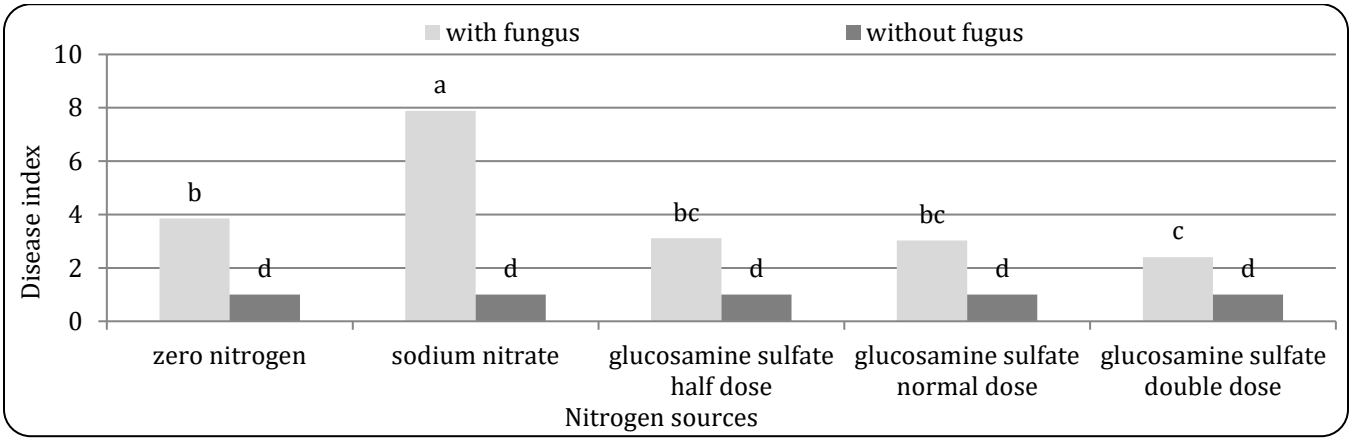

Figure 12. Effect of different amount of glucosamine sulfate on disease index of *Rhizoctonia solani* on faba bean plants under greenhouse conditions.

Table 1. Effect of different amount of glucosamine sulfate with fungus on plant growth characters of faba bean.

Nitrogen Sources	Stem length (cm)	Number of Leaves / Plant	Shoot Weight (g)	Root Weight (g)	Percentage of seedling emergence (%)
Zero nitrogen	29.6 ± 4.6 bc	4.9 ± 0.8 a	4.8 ± 1.8 bc	4.8 ± 0.6 b	81.4 ± 17.5 b
Sodium nitrate	29.3 ± 6.3 bc	4.8 ± 1.3 a	2.7 ± 09 d	2.8 ± 0.9 c	55.5 ± 16.6 c
Glucosamine sulfate half dose	28.4 ± 4.3 c	4.7 ± 1.0 a	4.9 ± 1.1 bc	6.9 ± 2.2 a	92.5 ± 14.7 ab
Glucosamine sulfate normal dose	29.4 ± 4.1 bc	4.8 ± 1.1 a	4.8± 0.8 bc	7.2 ± 1.8 a	92.5 ± 14.7 ab
Glucosamine sulfate double dose	29.6 ± 4.0 bc	4.8 ± 0.7 a	5.9 ± 1.8 a	7.1 ± 2.0 a	92.5 ± 14.7 ab

Data were subjected to analysis of variance (ANOVA). Numbers within a column followed by the same letters are not significantly different.

Table 2. Effect of different amount of glucosamine sulfate without fungus on plant growth characters of faba bean.

Nitrogen Sources	Stem length (cm)	Number of Leaves / Plant	Shoot Weight (g)	Root Weight (g)	Percentage of seedling emergence (%)
Zero nitrogen	31.7 ± 5.2 ab	4.8 ± 0.9 a	5.4 ± 1.6 ab	7.3 ± 1.7 a	100.0 ± 0.0 a
Sodium nitrate	32.2 ± 5.3 ab	4.6 ± 1.2 a	4.8 ± 1.4 bc	7.3 ± 1.9 a	100.0 ± 0.0 a
Glucosamine sulfate half dose	33.8 ± 5.5 a	4.9 ± 0.7 a	4.3 ± 1.4 c	7.4 ± 1.7 a	100.0 ± 0.0 a
Glucosamine sulfate normal dose	32.2 ± 4.3 ab	4.6 ± 0.5 a	4.2 ± 1.0 c	7.0 ± 1.9 a	100.0 ± 0.0 a
Glucosamine sulfate double dose	34.7 ± 4.7 a	5.0 ± 0.5 a	5.3 ± 1.1 ab	7.1 ± 1.7 a	100.0 ± 0.0 a
ANOVA *P* value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Data were subjected to analysis of variance (ANOVA). Numbers within a column followed by the same letters are not significantly different.

DISCUSSION

Nitrogen (N) considers one of the most vital components in dietary of all organisms included prokaryotic and eukaryotic organisms. Source of such vital compound ranged from atmospheric N_2 to mineral N as nitrites or nitrates amine or as ammonium salts. Organic compounds contain N as organic structure ranging from amino acids to polypeptides and proteins. *Rhizoctonia solani* can utilize all figures of N compounds except atmospheric N (Stephen and Fung, 1971). This fungus considers one of the soil borne phytopathogenic fungi, it does distribute in all cultivated soil causing very serious diseases include damping-off, root rot, stem canker and can cause the death of plants (Trivedi *et al.*, 2017). Since cultivated soil usually fertilized by different N sources includes nitrate, ammonium salts or organic amendments, then the fungal inoculum will feed either of these fertilizers which may affect its pathogenicity. The fungus usually survives in soil as sclerotia which germinate in the presence of suitable host to infect it. The fungus need for germination of sclerotia many nutrients needed for sclerotia growth (Moromizato *et al.*, 1980; Ritchie *et al.*, 2009).

The present study was passed through different ascending steps. The first one is studying the effect of nitrogen sources included mineral *i.e.* sodium nitrate, ammonium sulfate or simple organic *i.e.* glucosamine sulfate, phenylalanine, aspartic acid and protein *i.e.* peptone on fungal growth and its pathogenicity on faba bean seeds. The second one includes preparation of fungal inoculum on different mentioned sources in order to study the effect of them on fungal pathogenicity on faba bean plants. The third, the best nitrogen agent reduced the efficacy of the pathogen to infect faba bean plant was chosen for further study. Concerning the first step, ammonium sulfate led to stimulate fungal growth rate, contrary, glucosamine sulfate showed less growth rate compared with other nitrogen agents. The efficacy of such growth on disease severity on faba bean germinated seeds when such seeds were put directly on fungal growth indicated that growth of fungus on glucosamine sulfate significantly reduced its pathogenicity comparing with other tested N sources. Does such reduction was occurred due to the failing of growth mats grown on glucosamine sulfate to produce its infection cushions? From data obtained in the present study, a number of infection cushions on faba bean hypocotyls was significantly lower in case of glucosamine sulfate than that found in cases of other N sources. Infection cushions of *R. solani* is the first process of successful parasitism and their reduction will reflect on disease severity (Murray, 1982; Kim *et al.*, 2001).

Concerning the second step, the fungal inoculum was prepared on different nitrogen sources, data obtained showed that preparation of fungal inoculum on Czapek's medium contained glucosamine sulfate instead of sodium nitrate led the significant reduction of disease incidence in comparison with other agents. Does glucosamine sulfate can down regulate pathogenicity genes of *R. solani?* This point needs further study.

In the third step, glucosamine sulfate was tested in three concentrations *i.e.,* half dose, normal dose and double dose in comparison to sodium nitrate on fungal growth and its morphology, pathogenicity of fungal mats on faba bean germinated seeds, infection cushions and on polyphenol oxidase activity in infected seeds. Data obtained indicated that by increasing glucosamine sulfate in fungal medium growth was retained proportionally to increase of tested compound.

Concerning morphological features of fungal growth, it was noticed that presence of glucosamine sulfate in

fungal growth media led to the fungus failed completely in producing sclerotia, the main agent in the survival of the pathogen in soil. In this aspect (Moromizato *et al.*, 1980) have found that some sulfur containing amino acids inhibit the sclerotial formation of *R. solani*. Infection cushion was found to be less than that found on growth growing on Na_2NO_3. In the present study, ammonium sulfate as a sole source of nitrogen increased fungal linear growth also increased sclerotia formation. As mentioned, amino acids containing sulfur inhibit sclerotia to the obtained results this effect may not due to sulfur, since the fungus produced sclerotia well on ammonium sulfate. This fact was reflected in disease index. It decreased by increasing glucosamine sulfate concentration although, the seeds were directly put on fungal mats. The reason for decreasing disease index may due to the reduction of infection cushions which was obviously observed in this study. In order to ensure that disease index was decreased in increasing the glucosamine sulfate, polyphenol oxidase activity was determined hence, it was previously found that the activity of PPO increases by increasing disease severity (Shetty *et al.*, 2001; Parihar *et al.*, 2012). These results greatly indicate that glucosamine sulfate can affect the process of invasion during pathogenicity of such fungus due to retarding of pathogenicity gene (Lakshman *et al.*, 2012).

The last step in this investigation included the treatment of infested sand by different concentrations of glucosamine sulfate then seeded germinated faba bean seeds in such sandy soil. Sand was used for seeding faba bean seeds instead of natural soil to prevent any interference between glucosamine sulfate and other naturally found N sources in natural soil.

Data obtained indicated that glucosamine sulfate proved its efficacy on disease index which reduced by increasing glucosamine sulfate concentration. This beneficial effect was reflected in plant growth parameters *i.e.* seedling emergence, stem length, a number of leaves/plant, shoot weight and root weight. As a conclusion, glucosamine sulfate reduced *R. solani* growth, completely inhibit sclerotia production by the fungus, reduced disease index, and increased all parameters of plant growth.

REFERENCES

Bach, D. 1927. La nutrition azotée des Mucorinées. Assimilation de l'ion nitrique.

Duvenhage, J. A., J. M. Kotzé and E. M. C. Maas. 1992. The influence of nitrogen and calcium on mycelial growth and disease severity of *Phytophthora cinnamomi* and the effect of calcium on resistance of avocado to root rot. South African Avocado Growers' Association Yearbook, 15: 12-14.

Ghahramani, S. 2000. Fundamentals of probability Prentice Hall: New Jersey.

Huber, D. M. and R. D. Watson. 1974. Nitrogen Form and Plant Disease. Annual Review of Phytopathology, 12: 139-65.

Islam, R. 2015. Effect of various carbon and nitrogen sources on mycelial growth of *Fusarium* spp. isolated from agricultural fields of Murshidabad. Indian Journal of Scientific Research and Technology, 3: 71-77.

Jabin, F. and S. Nasreen. 2016. Phytochemical analysis of some medicinal plants. International Journal of Applied Research, 2: 293-95.

Kim, H.-T., Y.-R. Chung and K.-Y. Cho. 2001. Mycelial melanization of *Rhizoctonia solani* AG1 affecting pathogenicity in rice. The Plant Pathology Journal, 17: 210-15.

Lakshman, D. K., N. Alkharouf, D. P. Roberts, S. S. Natarajan and A. Mitra. 2012. Gene expression profiling of the plant pathogenic basidiomycetous fungus *Rhizoctonia solani* AG 4 reveals putative virulence factors. Mycologia, 104: 1020-35.

Littell, R. C., G. A. Milliken, W. W. Stroup and R. D. Wolfinger. 1996. SAS System for Mixed models. SAS Institute: Cary, NC.

Maha Helmy, M. 2015. Phenotypic diversity and molecular identification of the most prevalent anastomosis group of *Rhizoctonia solani* isolated from diseased faba bean plants. American Journal of Life Sciences, 3: 47.

Marzluf, G. A. 1997. Genetic regulation of nitrogen metabolism in the fungi. Microbiology and Molecular Biology Reviews, 61: 17-32.

Mohamed, M. H., E. A. M. Gado, S. H. El-Deeb and M. H. Mostafa. 2014. Effect of nitrate levels as a fertilizer or as a fungal nutrition on the aggressiveness of *Rhizoctonia solani* on faba bean. European Journal of Advanced Research in Biological and Life Sciences, 2: 1-13.

Moromizato, Z., N. Matsuyama and S. Wakimoto. 1980. The effect of amino acids on sclerotium formation of *Rhizoctonia solani* Kuehn (AG-1). I. Inhibition of sclerotial formation by various amino acids. Japanese Journal of Phytopathology, 46: 15-20.

Murray, D. I. L. 1982. Penetration of barley root and coleoptile surfaces by *Rhizoctonia solani*. Transactions of the British Mycological Society, 79: 354-60.

Parihar, P. S., O. Prakash and H. Punetha. 2012. Investigation on defensive enzymes activity of *Brassica juncea* genotypes during pathogenesis of alternaria blight. Nature and Science, 10: 63-68.

Ritchie, F., R. A. Bain and M. P. McQuilken. 2009. Effects of nutrient status, temperature and pH on mycelial growth, sclerotial production and germination of *Rhizoctonia solani* from potato. Journal of Plant Pathology: 589-96.

Ritter, G. 1909. Ammoniak und nitrate als stickstoffquelle für schimmelpilze.

Shetty, H. S., N. S. Vasanthi, B. R. Sarosh and K. R. Kini. 2001. Inheritance of downy mildew resistance, β-1,3-glucanases and peroxidases in pearl millet [*Pennisetum glaucum* (L.) R. Br.] crosses. TAG Theoretical and Applied Genetics, 102: 1221-26.

Stephen, R. C. and K. K. Fung. 1971. Nitrogen requirements of the fungal endophytes of *Arundina chinensis*. Canadian Journal of Botany, 49: 407-10.

Trivedi, A., S. K. Sharma, R. Chaudhary, D. K. Jajoria, R. Kumar Jain and S. K. Yadav. 2017. Management of dry root rot caused by *Rhizoctonia solani* in organic gram. International Journal of Current Microbiology and Applied Sciences, 6: 3647-52.

Ünal, M. Ü. 2007. Properties of polyphenol oxidase from anamur banana (*Musa cavendishii*). Food Chemistry, 100: 909-13.

White, C. and G. M. Gadd. 1983. Effect of glucosamine on morphology of *Verticillium alboatrum*. Transactions of the British Mycological Society, 80: 533-36.