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**By**

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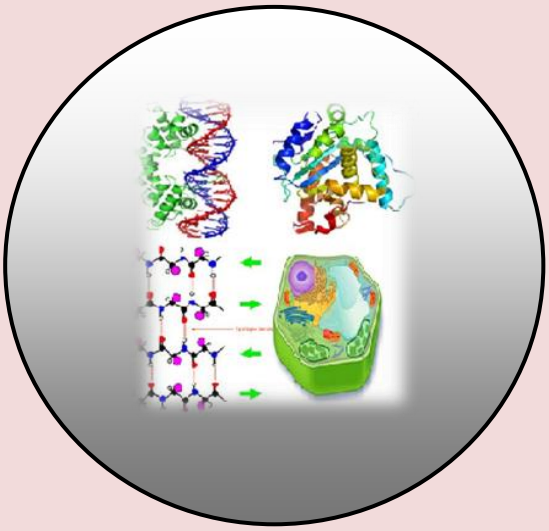

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# Effects of $SiO_2$ Nanoparticles and *Pochonia chlamydosporia* in Mitigating the Biotic Stress Induced by *Meloidogyne incognita* on Cowpea and Study of some Improved Plant Agronomic Attributes

**Atirah Tauseef, Mohd Danish and Hisamuddin**
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## ABSTRACT

*The deployment of Pochonia chlamydosporea is conceivably a non-chemical approach in the management of root-knot nematode (RKN), but its low persistency under field conditions restricts its use for their management. Addition of enhancer could boost the ability of the fungus to establish and inhibit roots consequently escalating its efficacy against the nematodes. For this purpose, a pot experiment was conducted under glass house conditions to assess the growth, physiology and yield characteristics of cowpea inoculated with Meloidogyne incognita. Fourteen day-old cowpea seedlings were supplied with two fungal suspension (10 and 20ml) and three doses of $SiO_2$ nanoparticles (50, 100, 200 ppm) alone and in combination with each other. The crop was harvested after 90 days of inoculation and its growth and yield parameters were recorded. The results depicted that 100 ppm of $SiO_2$ nanoparticles in combination of 20 ml of fungal culture showed the most significant and enhancing effects on growth and yield parameters viz. plant length, plant weight, number of pods per plant, number of seeds per pod, pod length and physiological parameters of plant in terms of chlorophyll a, b, carotenoids, nitrate reductase activity (NRA) and seed protein content in comparison to the inoculated control. All the concentrations of $SiO_2$ nanoparticles and P. chlamydosporia were potent enough to limit the number of galls, size of galls, egg masses per root system and root knot index and egg mass index. On the basis of the results it could be inferred that $SiO_2$ at 200 ppm along with P. chlamydosporea (20ml) would be efficient for M. incognita management.*

*Keywords- $SiO_2$ nanoparticles, Meloidogyne incognita and Pochonia chlamydosporea.*

## INTRODUCTION

Plant parasitic nematodes which infect a wide range of economically important crops causing 12% of reduction in yield resulting in a loss of about 125 billion dollors in the tropics (Chitwood 2003; Prabhu et al, 2009). Among the array of plant parasitic nematodes, Root-knot nematodes belonging to the genus *Meloidogyne* are aggressive sedentary endoparasites with extensive host range infecting almost all the crops of wild and cultivated origin including both monocots and dicots, putting more than 2000 plant species at risk (Moens et al, 2009; Sikora and Fernandez 2005; Wesemael et al, 2011; Fuller, et al, 2008).

The process of infection is commenced when second stage juveniles ingress inside roots near the zone of elongation, move towards the cortical region and vascular tissues where they choose four to eight cells as their feeding site and instigate aberrant cell division and growth culminating in multinucleate and hypertrophied giant cells. Moreover, hyperplasia of cells in the vicinity of giant cells leads to the formation of distinctly visible root knots- symptomatic characteristic of RKN infection (Gheysen and Mitchum, 2011 ; Kyndt, et al, 2014). However, other critical symptoms include stunted growth, chlorosis, wilting, necrosis (Pattison, 2007).

Until now, the foremost recurrent management strategies enforced for the control of nematodes are soil fumigants and nematicides. The hurdles associated with these nematicides is their low adequacy towards nematodes and potential contamination of environment such as ground water contamination. Furthermore, their appliance in particular countries have been restricted owing to their environmental hazards (Thoden et al, 2009).

The other means of suppressing nematodes is the usage of biological control agents (Ferraz et. al. 2010). The application of natural enemy of root-knot nematode *Pochonia chlamydosporia* has been investigated extensively (Larriba et al, 2014). Endophytic colonization of roots by *P. chlamydosporia* has a number of benefits to the host plant, such as growth promotion or protection against different pathogens such as nematodes and fungi (Maciá-Vicente, et. al. 2009; Monfort et al, 2005). Many studies insight *P. chlamydosporea* as a most tenable candidate for the efficient management of economically important plant parasitic nematodes infesting crop of agriculture importance (Khan et al, 2005; Viggiano et al, 2014; Deepa et al, 2011). Muthulakshmi et al, (2012) reported that *P.chlamydosporea* is integral part of IPM apporoach. Efficiency of bio-controlling agents has been ameliorated previously by boosting the capacity of microbial biological control agents to generate antimicrobial compounds namely antibiotics and hydrolytic enzymes (Bilal et al, 2017). Although, many reports cited the synergistic effect of different combinations of bio-controlling agents (bacteria and fungi) (Chemeltorit et al, 2017; Jambhulkar et al, 2018) but in accordance with review of publications examined by Xu et al, (2011), 98% of last studies on bio-controlling agents deploying microbial biological control agents combinations exhibited either little or no refinement in the performance of bio-control agents. The underlying fact may be the competitiveness or mutual antagonism within chosen microbes for space and nutrition.

Datnoff et al, (2007) reported that the inherent approach of enhancing defense in plants is via the application of non-essential elements such as Silicon and Aluminium. Silicon is the second most common present element on earth and occurs in plentiful amount in soil oxygen and has been recognized as a beneficial nutrient for the plant growth and development (Wainwright, 1997; Siddiqui et al, 2015). The applicability of Si known to impart resistance against many foliar and root invading pathogens (Datnoff et al, 2007). In this facet, a better apprehension of plant defence mechanism in response to nematodes is inexorable (Kyndt et al, 2014). Roots of coffee and cucumber accommodating silica in larger amount limits the nematode infection (Silva et al, 2010; Dugui et al, 2010). Keeping all these facets in view the present study was designed to deal with the different combination of biocontrol agent *P. chlamydosporea* and $SiO_2$ nanoparticles against *M. incognita.*

## MATERIAL AND METHOD

### NANOPARTICLES

#### $SiO_2$ nanoparticles

$SiO_2$ nanoparticles of 20 nm were purchased from Sigma Aldrich. Further the size of nanoparticles was confirmed by Transmission electron microscopy (TEM).

#### SiO2 nanoparticles suspension

Three different concentration of nanoparticles (50, 100, 200 ppm) were prepared by mixing respective amount of nanoparticles in deionized water.

#### Maintenance of nematode inoculam

From root-knot nematode infested fields of brinjal, roots with typical symptoms of galls were taken. Identification of *M. incognita* species was done in accordance with North Carolina differential host test and perineal pattern appearance. For establishment of pure culture of *M. incognita,* the egg plant was inoculated with a single egg mass and maintained under glass house conditions.

Later on, the plant was uprooted and egg masses were take out with the aid of sterilized forceps and were allowed to hatch followed by the collection of infective second stage juveniles ($J_2$) in autoclaved distilled water and the number of juveniles was counted under the stereomicroscope with the help of counting dish. The nematode suspension was standardized to 1,000 juveniles per 10 ml (Khan, 2008).

**Source of fungal culture**

Culture of *Pochonia chlamydosporia* (ITCC-6898) was procured from IARI, New Delhi. The fungus was sub-cultured on potato dextrose agar medium (Riker and R.S. Riker, 1936). The fungus was further mass cultured on potato dextrose broth medium kept in an incubator at $25^0$ C for 15 days. Afterwards, 100 gm mycelial mat was procured and shake with 1000 ml volume of double distilled water in an electric blender for 30 sec further volume is adjusted such that 10 ml of suspension consisted of one gram of mycelium. Accordingly, two different concentrations 10 ml and 20 ml were prepared and used in various combinations in different treatment fashion.

**Plant material and growth conditions**

Cowpea seeds (*Vigna unguiculata* L.var.gomti) were obtained from the Indian Agriculture Research Institute, New Delhi, India and stored in the dark at 6°C prior to use. Seeds were surface sterlized by immersing in 5% (w/v) sodium hypochlorite for 5 minutes followed by washing with double distilled water. The sterilized seeds were allowed to germinate in the dark at 25°C on moist filter paper. Three seedlings of 5- day- old per pot were maintained and total sixty five medium size pots of 25 cm filled with sterilized soil (sand 50%, silt 18%, clay 30%, pH 7.5, organic matter, 2%). The plants were regularly supplied with the water. For each treatment pattern, five biological replicates were used. Weeding was carried out whenever needed in order to keep the crop free from unwanted plants. Pre-germinated one week old cowpea seedlings were digged out and the roots were immersed in 20 ml of $SiO_2$ nanoparticle suspension of varying concentration for about 30 min. The roots of control plants were immersed in sterile water followed by the transfer of dipped roots in earthen pots containing sterilized soil and inoculated with 2000 $J_2$ of *M. incognita*

**Treatment pattern**

T1- *P. chlamydosporea* (10 ml)
T2- *P. chlamydosporea* (20ml)
T3- $SiO_2$ (50 ppm)
T4- $SiO_2$ (100 ppm)
T5- $SiO_2$ (200 ppm)
T6- $SiO_2$ (50 ppm) + *P. chlamydosporea* (10 ml)
T7- $SiO_2$ (100 ppm) + *P. chlamydosporea* (10ml)
T8- $SiO_2$ (200 ppm) + *P. chlamydosporea* (10ml)
T9- $SiO_2$ (50 ppm) + *P. chlamydosporea* (20ml)
T10- $SiO_2$ (100 ppm) + *P. chlamydosporea* (20ml)
T11- $SiO_2$ (200ppm) + *P. chlamydosporea* (20ml)

**Studied characteristics**

**Growth and weight measurements**

The crop was harvested after 90 days of transplantation. The lengths of shoots and the roots of cowpea plants was recorded. The plants were thoroughly washed with distilled water followed by drying with blotting sheets and weighed with the help of electronic balance (fresh weight). The plants were wrapped in paper bag and dried at $60^0$ C for 5 days and then dry weight measurements were made.

**Photosynthetic pigments measurement.**

Estimation of chlorophyll and carotenoids was done in accordance with the method described by G. McKinney (1941). Extraction of chlorophyll and carotenoids from leaves of each replicate using 80% acetone was performed.

**Nitrate reductase Activity**

The nitrate reductase activity was recorded using the method described by Jaworski, (1971). Phosphate buffer of 0.1 M was prepared, and 1.25 ml poured into each of sixty five test tube in which freshly chopped leaves (100mg) were added. In each test tube, 0.2 M potassium nitrate (0.25ml) and 5% isopropanol (1.25 ml) was added followed by transferring the mixtures in BOD incubator maintained at 25±2 $^0$ C for 2 hours.

Later on, in each test tube 1% sulphanilamide (0.15 ml) and 0.02% NED-HCL was added. The resultant solutions were kept at room temperature for 20 minutes until the development of reddish pink color. The resulting mixture was subjected to dilution with double distilled water to maintain 5ml volume. The absorbance was noted at 540 nm against blank by making use of spectrophotometer followed by curve plotting using known concentration of sodium nitrite.

**Seed protein content**

Cowpea seed content was estimated by the method described by Lowry et al, (1951). The appearance of blue color indicated that the aromatic amino acids tryptophan and tyrosine present in protein reduces the Phosphomolybdic-phosphotungentic constituents of the Folin-Ciocalteu reagent, in addition color also appeared due to biuret reaction in which protein reacts alkaline cupric tartarate as estimated in accordance with Lowry method that can be assessed by noting absorbance at 660 nm by deploying spectrophotometer.

**Number and size of galls**

The number of galls per plant were counted visually. Dimensions of galls (length and width in mm) was measured by using micrometer.

**Number of egg masses per root system**

The number of egg masses per root was obtained in accordance with the methodology of Holbrook et al, (1983). Staining of infected roots was done by using phloxin-B by keeping them in solution for about 20 minutes. Under running tap water, the roots were rinsed smoothly followed by counting of stained egg masses.

**RKI and EMI**

Galling index and egg mass index was recorded in accordance with the scale described by Taylor and Sasser (1978) where 0 represents no galling, 1 represents 1-2 galls/root , 2 refers to 3-10 galls/ root , 3 refers to 11-30 galls/ root, 4 pinpoints 31-100 galls/root and 5 refers to more than 100 galls/root system.

**Statistical analysis**

The recorded data were statistically analyzed by SPSS (version 20) and significance of variance was calculated at $P \leq 0.05$ level of probability.

## RESULTS AND DISCUSSION

The present experiment was carried out to investigate the effects of bio-controlling fungus- *P. chlamydosporea* and $SiO_2$ nanoparticles alone and in different combinations with each other on agronomic and biochemical attributes of cowpea infected with *M. incognita.* The growth of plant in terms of shoot length and root length, weight (fresh, dry) was analyzed. The results clearly revealed that all the treatments (*P. chlamydosporea* and $SiO_2$ nanoparticles separately and concomitantly) improved the growth of plants and proved antagonistic to *M. incognita*. The results obtained depicted that *M. incognita* inoculation was deleterious as infection had drastically altered the morphology and physiology of cowpea. Retarded growth of infected plants was due to unavailability of nutrients in sufficient amount. It could be attributed to the fact that root-knot nematode hampers the process of nutrient and water uptake, mobilization of photosynthates and nutrients (Williamson and Hussey, 1996). Anwar and Van Gundy (1989) reported that *M.incognita* infestation changes the root to shoot ratio. Siddiqui et al, (2014) reported that root-knot infestation leads to the establishment of giant cells from where the nematode feeds obtain its nutrition resulting in retardation of root growth and swelling of root tips ultimately declining the root and the shoot length. Babu et al, (2009) reported diagnostic characters of root-knot disease such as gall formation, chlorosis, loss of yield and dwarfing. On application of biocontrolling nematophagous the fungus *P. chlamydosporea*, development of root-knot nematode was found to be limited due to aggressive parasitizing behavior of fungus. In different doses (10 and 20 ml), the fungus causes significant reduction in number of galls, number of egg masses, size of galls over inoculated control (Table1). However, greater reductions of 49.14%, 25.68% and 37.92% in number of galls, size of galls, number of egg masses per root system, respectively were observed in plants supplied with higher dose (20ml) of the fungus. It was presumed that on application of higher dose of *P. chlamydosporea*, the fungus would grow over the large area of most of the roots thereby increasing the surface area for better absorption of nutrients required for the growth and development of the plants.

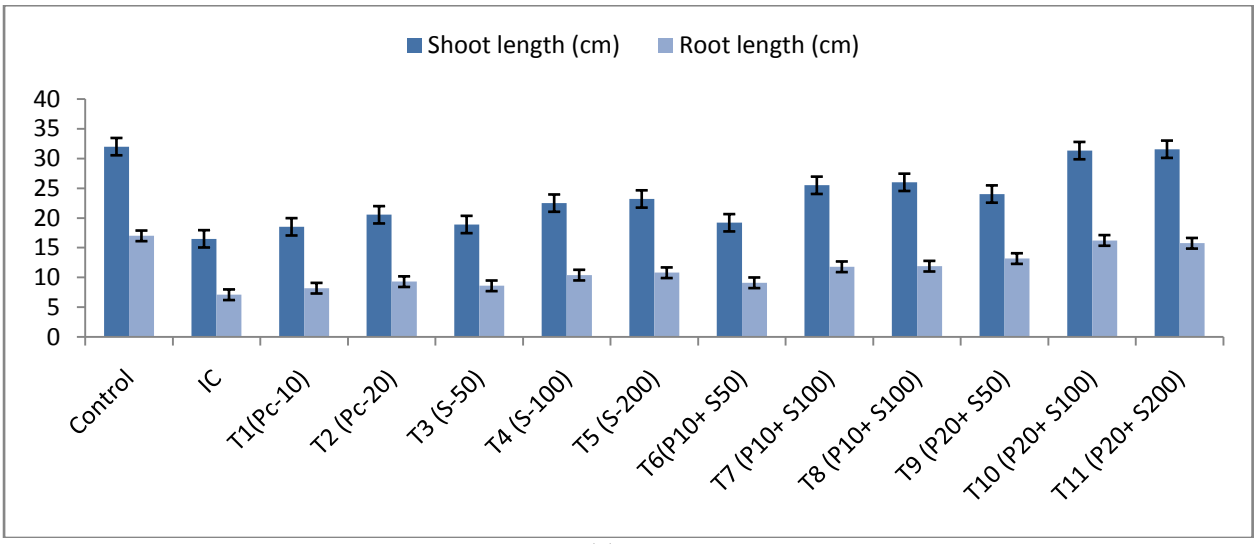
*P. chlamydosporea* being a facultative parasite of *Meloidogyne spp*. parasitizes both eggs and unveiled females of the nematode, and posses the enormous potential of generating abundant chlamydospores which are invulnerable to environmental stress thereby enabling the survival of this fungus during unfavorable conditions in the soil (Dallemole-Giaretta et al, 2012; Yang et al, 2012). Kerry (2000) reported that parasitizing behavior of fungus was due to the secreted enzymes that break down the egg coverings of diverse plant parasitic nematodes. Many studies insights *P. chlamydosporea* as a most tenable candidate for the efficient management of economically important plant parasitic nematodes infesting crop of agriculture value (Khan et al, 2005; Viggiano et al, 2014; Deepa et al, 2011). Muthulakshmi, et al. (2012) reported that *P. chlamydosporea* is integral part of IPM approach.

In the treatments T1 and T2 (10 and 20 ml) besides suppressive effects of *P. chlamydosporea* against root-knot nematodes, growth promoting effects of the fungus were also recorded. The decline in nematode population culminated in better growth of plants. Lengths of plants (shoot, root), fresh and dry weights of roots and shoots were increased significantly by 24.48%, 30.98%, 22.39%, 33.10%, 34.30% respectively in *P.chlamydosporea* treated plants over the inoculated control. Similar trend in yield parameters such as number of pods per plant (53.18%), number of seeds per pod (36.44%), pod length (28.18%) and seed weight (20.80%) was also observed. Yield improvement of nematode infected crops such as cotton, chilli, lemon, lettuce, brinjal on *P. chlamydosporea* application has been documented by many workers (Wang et al, 2005; Singh et al, 2011; Deepa et al, 2011; Dias-Arieira et al, 2011; khan et al, 2012). *P. chlamydosporea* being a root endophyte, ameliorate growth of a range of host plant species and sustaining their defense reaction to different pathogens (Maciá-Vicente et al, 2009; Ciancio et al, 2013).

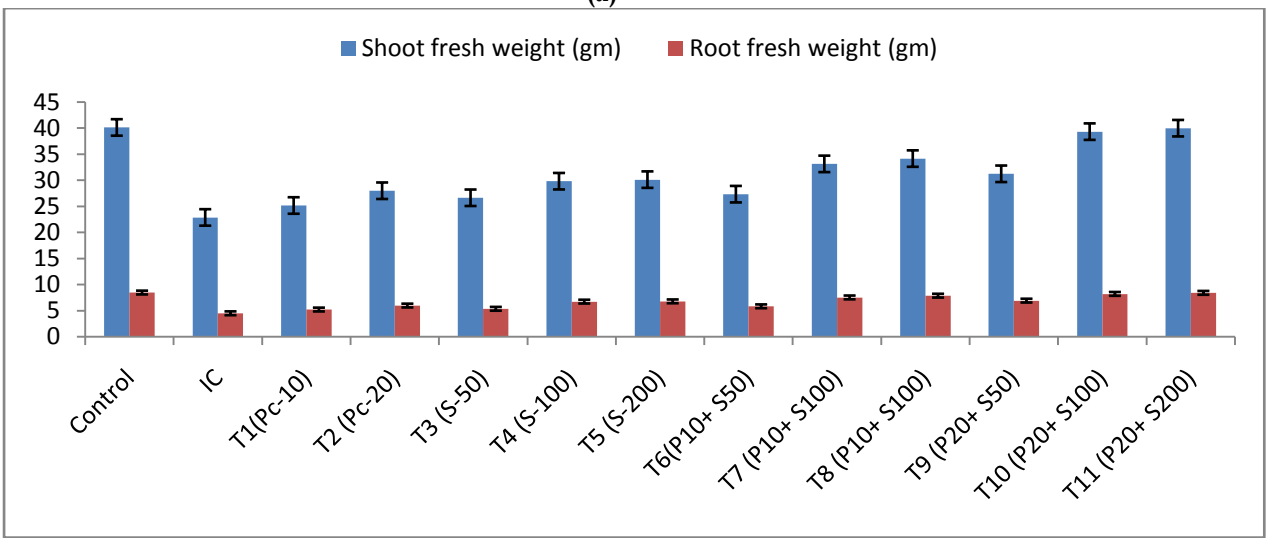
The plants treated with 50, 100, 200 ppm concentration of $SiO_2$ nanoparticles (T3, T4, T5) were tested against *M.incognita* infecting cowpea, nematicidal effects of $SiO_2$ nanoparticles were noticed. The results clearly revealed higher the concentration applied, greater is the nematicidal effects. In T3 treatment (50 ppm) concentration, significant reduction of (45.30%) in number of galls, size of galls (15.78%), number of egg masses (35.81%) was observed. However at higher concentration of 100 ppm (T4) and 200 ppm (T5) $SiO_2$ nanoparticles, reduction was comparatively higher (Table1). Ardakani (2013) investigated the nematotoxicity nature of Ag, $SiO_2$ and $TiO_2$ nanoparticles on second-stage juveniles ($J_2$) of the root-knot nematode, *M. incognita,* in laboratory experiments. In this experiment, it was seen that all treatments of AgNP and 0.02 % $TiO_2$ NP completely controlled *M. incognita.* Pluskota et al, (2009) reported that the Silica nanoparticles were capable of inducing degeneration of reproductive organs in *Caenorhabditis elegans.* It was reported that the mortality rate of invasive larvae of entomopathogenic nematodes depended on the concentration and the time of exposure to nanoparticles (Kucharska and Pezowicz 2009; Kucharska et al, 2011). The NPs cause different physiological and morphological changes in the plants (Khodakovskaya et al, 2012) and efficacy of NPs to cause these changes is widely determined by composition, concentration , size, physical and chemical properties of NPs and genetic properties of plant species (Ma, et. al. 2010).

The application of different concentration of $SiO_2$ nanoparticles not only limited nematodes reproduction but also promoted the vegetative growth and yield and physiological parameters (Fig.1, 2, 3). All the parameters showed significant increase over inoculated control. The results from current experiment revealed that plants treated with 200 ppm of Silicon dioxide nanoparticles, plant biochemical attributes such as chlorophyll a, b ( 19.78%, 26.56%) content, carotenoid content (20.25%), seed protein content (17.72%) , NRA (14.63%) increases significantly over the inoculated control. It has been revealed that exogenous application of nano-silicon on plants enhances the plant growth and development by increasing accumulation of proline, free amino acids, content of nutrients, antioxidant enzymes activity, gas exchange and improve efficiency of photosynthetic apparatus (Xie et al, 2012; Kalteh et al, 2014). Lu et al **(**2002) have shown that a combination of nano-sized $SiO_2$ and $TiO_2$ could increase nitrate reductase enzyme in soybean (*Glycine max*) increases its abilities in absorbing and utilizing water and fertilizer, encourage its antioxidant system, and actually hastens its germination and growth. Even though, many reports cited the complicated interaction of *P. chlamydosporea* with biotic and abiotic components that can govern the antagonistic ability of the fungus. Consequently, it is mandatory to recognize the components that influence virulence capability, growth and development of *P. chlamydosporea* (Esteves et al, 2009).

Rhizosphere colonization by bio-controlling fungi is the key factor for nematode control rather than fungal abundance as abundance is not always consonant with nematode egg parasitism (Kerry and Hirsch, 2011). In the combined application of $SiO_2$ nanoparticles and *P. chlamydosporea* the results procured were more promiscuous. From the results it could be recommended that nematicidal effects displayed by the $SiO_2$ nanoparticles and *P. chlamydosporea* were enhanced, which is responsible for greater nematode mortality and escalated plant growth. Silicon fertilization promotes the absorption of potassium and restricts the absorption of sodium, which therefore increases potassium/sodium selection ratio, helping the accumulation of potassium, nitrogen and sulphur in plants, and improving plant nutrition. In addition to the impact of Si on plant protection, various other beneficial effects of Si have been reported, such as amelioration of the adverse effects of Al and Mn toxicity to plants, improvement of water use efficiency (Guo, 2000; Hu and Schmidhalter, 2005). Tahir et al, (2010) reported that silicon application significantly increased wheat biomass at both control as well as under saline conditions.




**(a)**

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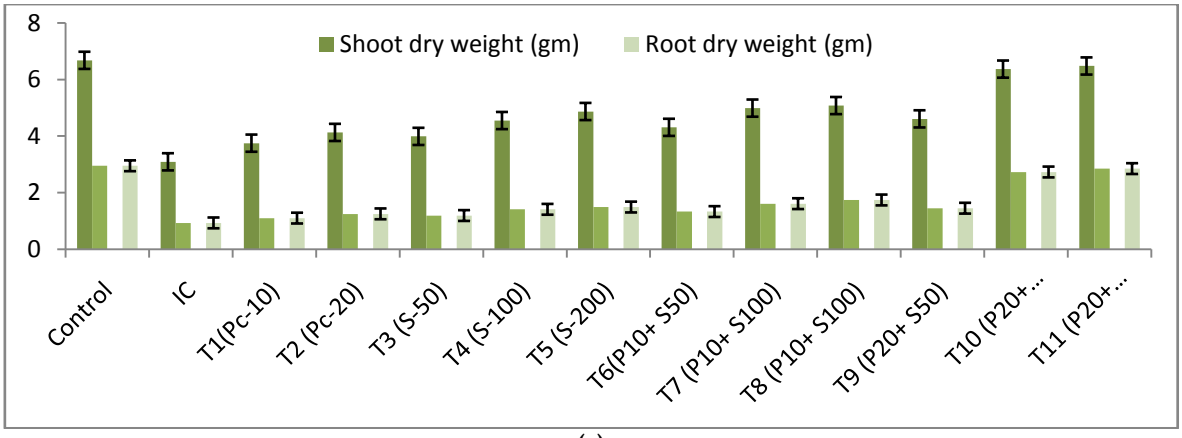
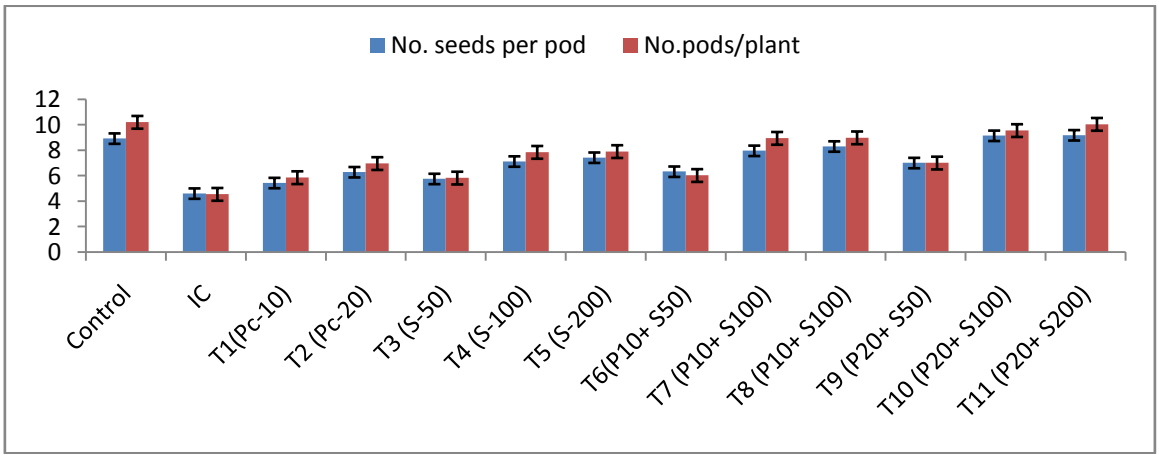



**(c)**

**Figure 1. Effects of different doses of *Pochonia chlamydosporea* and Silicon dioxide ($SiO_2$) nanoparticles alone and concomitantly on morphological attributes (a) shoot and root length (b) shoot and root fresh weight (c) shoot and root dry weight of infested cowpea. Data are the average of five replicates and vertical bar represents standard error. IC: plants inoculated with *M. incognita*, Pc: Plants treated with *P. chlamydosporea*, S: $SiO_2$ nanoparticles.**

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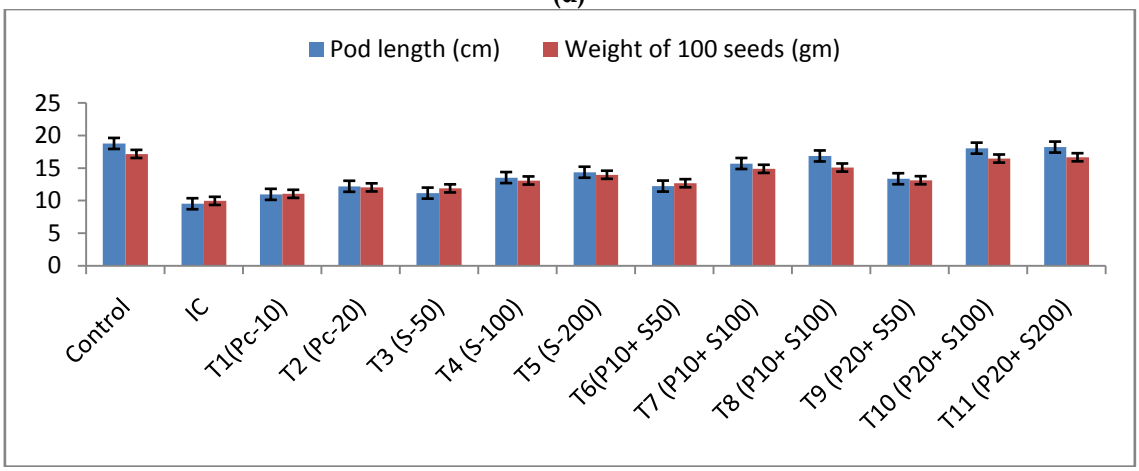
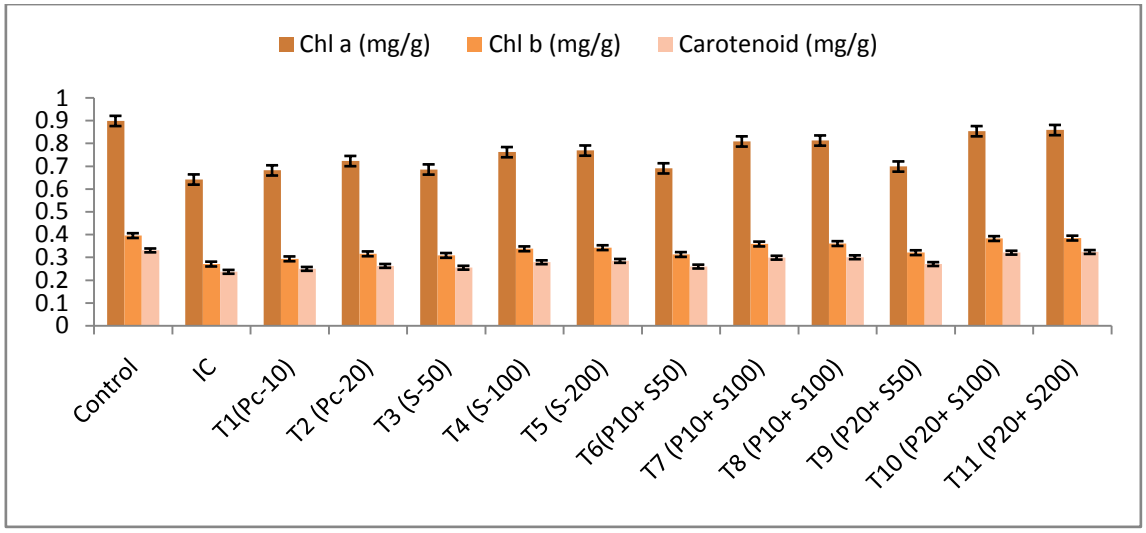



**(e)**

**Figure 2. Effects of *P.chlamydosporea* and $SiO_2$ nanoparticles alone and concomitantly on yield parameters (d) number of pods per plant and number of seeds per pod (e) pod length and weight of 100 seeds. Data are the average of five replicates and vertical bar represents standard error. IC: plants inoculated with *M.incognita*, Pc: Plants treated with *P. chlamydosporea*, S: $SiO_2$ nanoparticles.**

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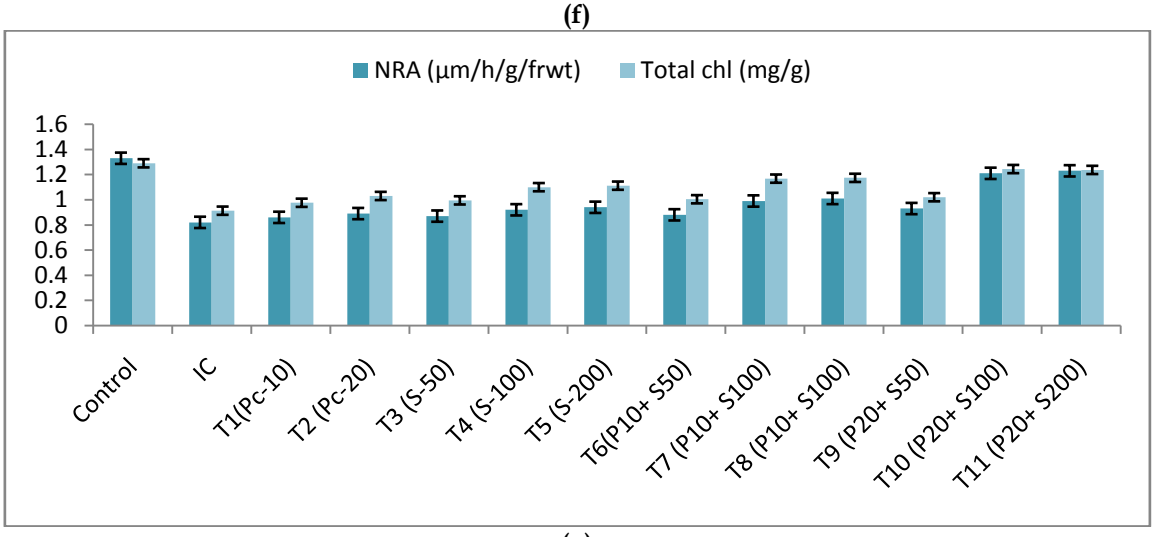


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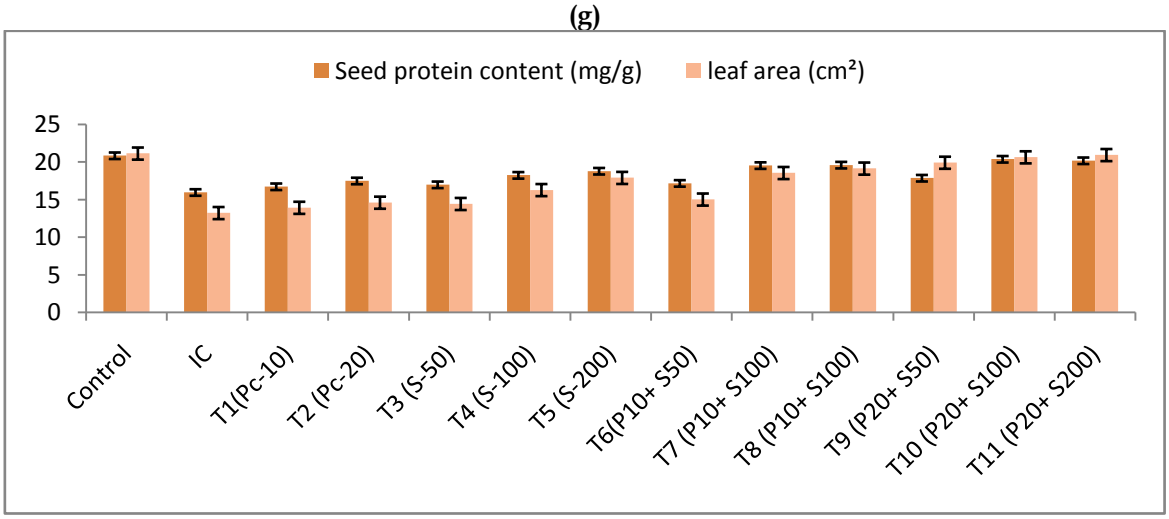


**(h)**

**Figure 3. Effects of *P. chlamydosporea* and $SiO_2$ nanoparticles alone and concomitantly on biochemical parameters (f) chlorophyll a, b and carotenoid (g) NRA and total chlorophyll (h) seed protein content and leaf area. Data are the average of five replicates and vertical bar represents standard error. IC: plants inoculated with *M.incognita*, Pc: Plants treated with *Pochonia chlamydosporea*, S: Silicon dioxide nanoparticles.**

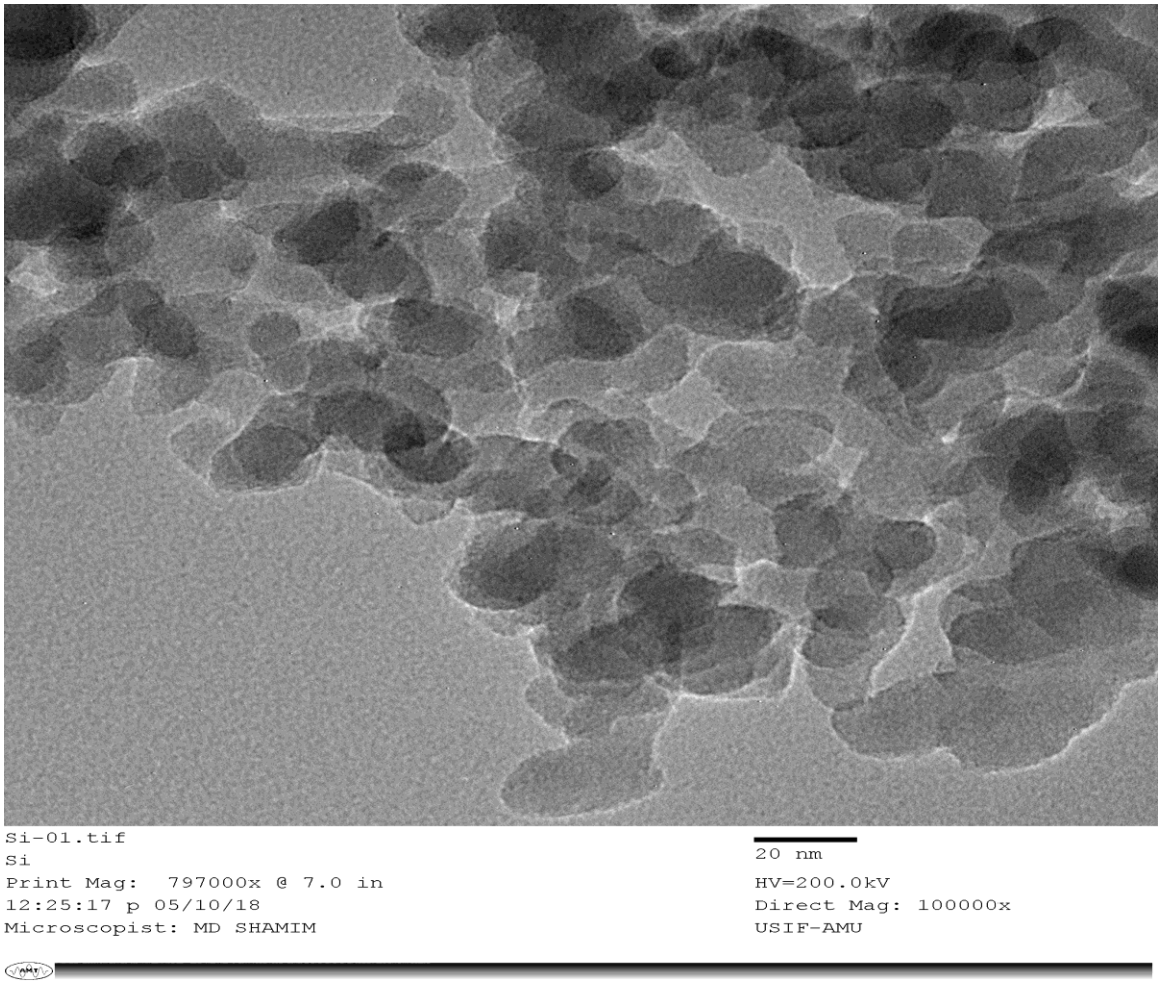


**Figure 4. TEM of $SiO_2$ nanoparticles.**

**Table 1. Effects of *Pochonia chlamydosporea* and Silicon dioxide nanoparticles alone and concomitantly on the number of galls, size of galls and number of egg masses of RKN infesting cowpea**

| Parametres/ Treatments | No. galls/ roots | Size of galls ($mm^2$) | No. egg masses/ root system | EMI | RKI |
|---|---|---|---|---|---|
| **Control** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **IC** | 100.8 | 19.00 | 102.54 | 5.00 | 5.00 |
| **Pc 10ml** | 57.95 | 16.50 | 63.65 | 4.00 | 4.00 |
| **Pc 20ml** | 51.26 | 14.12 | 55.14 | 4.00 | 4.00 |
| **$SiO_2$ 50ppm** | 55.13 | 16.00 | 65.82 | 4.00 | 4.00 |
| **$SiO_2$ 100 ppm** | 43.54 | 12.65 | 47.42 | 4.00 | 4.00 |
| **$SiO_2$ 200ppm** | 29.64 | 11.90 | 28.29 | 3.00 | 4.00 |
| **Pc10+$SiO_2$50** | 53.23 | 15.40 | 31.31 | 4.00 | 4.00 |
| **Pc10+$SiO_2$100** | 9.10 | 14.00 | 14.00 | 2.00 | 3.00 |
| **Pc10+$SiO_2$200** | 8.03 | 10.00 | 9.16 | 2.00 | 2.00 |
| **Pc20+$SiO_2$50** | 31.31 | 14.72 | 30.23 | 4.00 | 4.00 |
| **Pc20+$SiO_2$100** | 1.92 | 3.56 | 1.94 | 1.00 | 1.00 |
| **Pc20+$SiO_2$200** | 1.32 | 3.95 | 1.87 | 1.00 | 1.00 |
| **L.S.D** | 5.42 | 1.04 | 8.13 | - | - |

Data are Mean ± SE (n=5). Probablities of significance among the treatments and means were compared with the least significant differences (LSD) (P≤0.05). IC: plants Inoculted Control, Pc: *Pochonia chlamydosporea*, $SiO_2$: Silicon dioxide nanoparticles

In T9, T10 and T11 treatments, in which double dose of *P. chlamydosporea* (20ml) was applied with three different concentrations (50, 100, 200 ppm) of $SiO_2$ nanoparticles, the maximum reduction in size and number of galls, number of egg masses per root system was encountered in T11 (*P. chlamydosporea*-20ml+200 ppm of $SiO_2$ nanoparticles) plants followed by T10 which could reasonably be lesser number of juveniles penetration inside the roots thereby diminishing disease severity. Deposition of lignin in cell walls of host plant could be the attributed reason for hindrance of nematode penetration. Lignin is synthesized via phenylpropanoid route during which a number of phenolics for instance flavonoids, plant harmones, phytoelexins were formed as side products (Kim and Hwang, 2014). Lignin accumulation retards the nematode penetration directly by acting as a physical barrier and by making cell walls resistant to enzymes secreted nematodes (Gheysen and Jones, 2006). Moreover, Wuyts et al, (2006) reported that in case of *Arabidopsis thaliana*, higher level of syringyl lignin retards the *M. incognita* activity. In these sequentially treated plants, the fungus and nanoparticles caused significant reduction in retrogressing effect of *M. incognita* and thereby emanating improved environmental conditions for the growth of plants influencing morphological, physiological and yield attributes. From the results it could be inferred that $SiO_2$ nanoparticles played twin role, primarily in the form of lignin deposition on the plant cell wall which resist the *M. incognita* invasion and secondly by increasing the fungal population colonizing root surface thereby decreasing the penetration of root–knot juveniles culminating in lesser number of galls. Karunakaran et al, (2014) reported that Silicon addition in nano-dimension and bulk form escalates microbial population of plant growth promoting rhizobacteria. Wainwright et al, (2003) reported that population of microbes enhances upon the silicon addition. Therefore, in the presence of PGPR, amount of soil nutrients was increased, resulting in growth and yield promotion. Wu et al, (2005) reported that characteristics of soil and composition of mineral nutrients in particular nitrogen, phosphorus and potassium showed melioration in the presence of *Azotobacter*, *Bacillus megaterium*. *P. chlamydosporea*, possesses potential to kill the females as well as egg masses of root-knot nematode. The deadly action of fungus and $SiO_2$ nanoparticles is deduced to be responsible for betterment of plant growth characters. The soil environment in the vicinity of roots was made healthful by the rhizosphere colonization of fungus and was in accordance with plant nutrient demand. Consequently, yield parameters of cowpea such as number of pods per plant, number of seeds per pod, pod length was escalated ensuring nutrient supply. Due to endophytic nature of *P. chlamydosporea*, the fungus penetrated the inner lying tissues favorably and anastomosed in the entire root tissue without making cellular alterations. The lengths of plants, fresh and dry weights of roots and shoots exhibited greater improvement over the individual treatments. Suriyaprabha et al, (2015) studied the interaction of silicon nanotubes on maize and found that its application of low doses enhanced seed germination along with improved nutrient uptake. It has been observed that silicon plays pivotal role in enhancing physiological attributes of plants, escalating plant growth, instigating resistance against biotic and abiotic stresses (Kanto et al, 2004). Lahrmann et al, (2015) while working on interaction of sebacinoid fungi with *Arabidopsis thaliana* reported that fungal application escalates plant growth, floral development and seed productivity. Besides growth promotion, fungal endophytes also played role in ameliorating seed germination, root development by generating auxins (Chutima and Lumyong, 2012).

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## REFRENCES

**Anwar, S.A. and Van Gundy, S.D. (1989).** Influence of four nematodes on root and shoot parameters in grapes. *Nematol*. 21. 276-283.

**Ardakani, A.S. (2013).** Toxicity of silver, titanium and silicon nanoparticles on the root-knot nematode, *Meloidogyne incognita* and growth parameters of tomato. Nematol. 15 (6). 671–677.**Babu, A.M., Kumar, V. and Philip, T. (1999).** Root-Knot nematode- A hard to kill parasitic study. *Indian Silk* 38. 11-12.

**Bilal, M., Guo, S., Iqbal, H.M.N., Hu, H., Wang, W. and Zhang, X. (2017).** Engineering Pseudomonas for phenazine biosynthesis, regulation, and biotechnological applications: a review. *World J Microbiol Biotechnol.* 33. 191.

**Chemeltorit, P.P., Mutaqin, K.H. and Widodo, W. (2017).** Combining *Trichoderma hamatum* THSW13 and *Pseudomonas aeruginosa* BJ10–86: a synergistic chili pepper seed treatment for *Phytophthora capsici* infested soil. *Eur J Plant Pathol*. 147. 157–166.

**Chitwood, D.J .(2003).** Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture–Agricultural Research Service. Pest Manag Sci . 59 (6-7). 748–753

**Chutima, R. and Lumyong, S. (2012).** Production of indole-3-acetic acid by Thai native orchid-associated fungi. Symbiosis 56, 35-44.

**Ciancio, A., Colagiero, M., Ferrara, M., Nigro, F., Pentimone, I. and Rosso, L.C. (2013).** Transcriptome changes in tomato roots during colonization by the endophytic fungus *Pochonia chlamydosporia*. Abstr. 5th Congress, Federation of the European Microbiologists Societies (FEMS), July 21–25, Leipzig, Germany.

**Dallemole-Giaretta, R., Freitas, L.G., Lopes, E.A., Pereira, O.L., Zooca ,R.J.F. and Ferraz, S. (2012).** Screening of *Pochonia chlamydosporia* Brazilian isolates as biocontrol agents of *Meloidogyne javanica* Crop Prot.42. 102-107.

**Datnoff, L.E., Rodrigues, F.Á. and Seebold, K.W. (2007).** Silicon and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer, DM Huber) pp. 233–246. (The American Phytopathological Society Press: St Paul, MN).

**Deepa, S.P., Subramanian, S. and Ramakrishan, S. (2011).** Biomanagement of citrus nematode *Tylenchulus semipenetrans* Cobb on lemon, *Citrus limonia* L. J. Biopest. 4. 205–207.

**Dias-Arieira, C.R., Santana, S.M., de Freitas, L.G., da Cunha,T.P.L., Biela, F., Puerari, H.H. and Chiamolera, F.M. (2011).** Efficiency of *Pochonia chlamydosporia* in *Meloidogyne incognita* control in lettuce crop (Lactuca sativa L.). J. Food, Agri and Environ. 9. 561–563.

**Dugui, Es.C., Pedroche, N., Villanueva, L., Galeng, J. and De Waele, D. (2010).** Management of root knot nematode, *Meloidogyne incognita* in cucumber (*Cucumis sativus*) using silicon. *Commun Agric Appl Biol Sci.* 75. 497-505.

**Esteves, I., Peteira, B., Atkins, S., Magan, N. and Kerry, B. (2009).** Production of extracellular enzymes in different isolates of *Pochonia chlamydosporia*. *Mycol. Res.* 113. 867-876.

**Ferraz, Freitas,S .L.G. de., Lopes, E.A. and Dias-Arieira e, C.R. (2010).** Manejo sustentável de fito nematoides. 304 p. Editora UFV, Viçosa, Brasil.

**Fuller, V.L., Lilley, C.J. and Urwin, P.E. (2008.)** Nematode resistance. New Phytol. 180. 27–44. doi:10.1111/j.1469-8137.2008.02508.x

**Gheysen, G. and Jones, J. (2006).** In: Plant Nematology ( Perry, R.N. and Moens, M., eds), Molecular aspects of plant nematode interaction. Wallingford, Oxfordshire: CAB International. Pp. 234-254.

**Gheysen, G. and Mitchum, M.G. (2011).** How nematodes manipulate plant development pathways for infection. *Curr Opin Plant Biol.* 14. 415– 421.

**Guo, Z. (2000).** Synthesis of the needle-like silica nanoparticles by biomineral method. *Chem J Chinese* U. 21 (6). 847–848.

**Holbrook, C.C., Knauft, D.A. and Dikson, D.W. (1983).** A technique for screening peanut for resistance to *Meloidogyne arenaria*. *Plant Dis.* 57. 957–958.

**Hu, Y. and Schmidhalter, U. (2005).** Drought and salinity: A comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci.* 168. 541–549. doi: 10.1002/jpln.200420516.

**Jambhulkar, P.P., Sharma, P., Manokaran, R., Lakshman, D.K., Rokadia, P. and Jambhulkar, N. (2018).** Assessing synergism of combined applications of *Trichoderma harzianum* and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice. Eur J Plant Pathol. 152. 747–757.

**Jaworski, E.G. (1971).** Nitrate reductase assay in intact plant tissue. *Biochem. Bioph. Res. Co.* 43. 1274-1279.

**Kalteh, M., Alipour , Z.T., Ashraf, S., Aliabadi, M.M. and Nosratabadi, A.F. (2014).** Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress. *J. Chem. Health Risks.* 4(3). 49–55.

**Kanto, T., Miyoshi ,A., Ogawa, T., Maekawa, K. and Aino, M. (2004).** Suppressive effects of potassium silicate on powdery mildew of strawberry in hydroponics. *J Gen plant pathol*. 70. 207-211.

**Karunakaran, G., Suriyaprabha, R., Manivasakan, P., Rajendran, V. and Kannan, N. (2014).** Influence of nano and bulk SiO2 and AL2O3 particles on PGPR and soil nutrient contents. *Curr. Nanosci.* 10 (4). 604-612.

**Kerry, B.R. (2000).** Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant- parasitic nematodes. *Annu. Rev. Phytopathol.* 38. 423-441.

**Kerry, B.R. and Hirsch, R.R (2011).** Ecology of Pochonia chlamydosporia in the rhizosphere at the population whole organism and molecular scales. In: Biological Control of Plant- Parasitic Nematodes: Building Coherence between Microbial Ecology and Molecular Mechanisms (K.G. Davies, Y. Spiegel, ed). Springer, London, UK, 171-182.

**Khan, M.R. (2008).** In: Methodology, Morphology, systematics, Biology and Ecology . Plant nematodes. New Hampshire, Science Publishers. USA.

**Khan, M.R., Khan, S.M. and Mohide, F. (2005)** Root-knot nematode problem of some winter ornamental plants and its biomanagement. *J. Nematol.* 37. 198–206.

**Khan, M.R., Zaidi, B. and Haque, Z. (2012).** Nematicides control rice root-knot, caused by *Meloidogyne graminicola*. *Phytopathol. Mediterr.* 51. 298-306.

**Khodakovskaya, M.V., Silva, K de., Biris, A.S., Dervishi, E. and Villagarcia, H. (2012).** Carbon nanotubes induce growth enhancement of tobacco cells. *Nano*. 3. 2128–2135.

**Kim, D.S. and Hwang, B.K. (2014).** An important role of the pepper phenylalanine ammonia lyase gene (PALI) in salicylic acid- dependent signaling of the defense response to microbial pathogens. *J. Exp. Bot.* 65. 2295-2306.

**Kucharska, K. and Pezowicz, E. (2009).** The effect of silver nanoparticles on mortality and pathogenicity of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) from Nematop biopreparation. Artykuły IV Międzynarodowej Konferencji Doktorantów i Młodych Naukowców "Young scientists towards the challenges of modern technology", Warszawa, Wrzesien 21-23.

**Kucharska, K., Tumialis, D., Pezowicz, E. and Skrzecz, I. (2011).** The effect of gold nanoparticles on the mortality and pathogenicity of entomopathogenic nematodes from Owinema biopreparation. Insect pathogens and entomopathogenic nematodes IOBC/wprs Bulletin 66. 347–349.

**Kyndt, T., Fernandez, D. and Gheysen, G. (2014).** Plant-parasitic nematode infections in rice: molecular and cellular insights. *Annu Rev. Phytopathol.* 52. 135–153.

**Lahrmann, U., Strehmel ,N., Langen, G., Frerigmann, H., Leson, L., Ding, Y., Scheel ,D., Herklotz, S., Hilbert, M. and Zuccaro, A. (2015).** Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a non compromised plant innate immunity. New Phytol. 207. 841–857.

**Larriba, E. et al.** Sequencing and functional analysis of the genome of a nematode egg-parasitic fungus, *Pochonia chlamydosporia*. *Fungal Genet. Biol.* 65, 69–80 (2014).

**Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951).** Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193. 265–275.

**Lu, C.M., Zhang, C.Y., Wu, J.Q. and Tao, M.X. (2002).** Research of the effect of nanometer on germination and growth enhancement of *Glycine max* and its mechanism. *Soybean Science* 21. 168–172.

**Ma, X., Geiser-Lee, J., Deng, Y. and Kolmakov, A. (2010).** Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Sci Total Environ*. 16. 3053–3061.

**Maciá-Vicente, J.G., Rosso, L.C., Ciancio, A., Jansson, H.B. and Lopez-Llorca, L.V. (2009b)** Colonisation of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosporia*: Effects on plant growth and disease. *Ann Appl Biol.* 155 (3). 391–401.

**McKinney, G. (1941).** Absorption of light by chlorophyll solutions. *J. Biol. Chem.* 140. 315–322.

**Moens, M., Perry, R.N. and Starr, J.L. (2009).** Meloidogyne species– a diverse novel group and important plant parasites. In: Moens M, Starr JL (eds) Perry, R.N. CAB International, Root-knot nematodes. Wallingford. UK pp 1–17.

**Monfort, E., Lopez-Llorca, L.V., Jansson, H.B., Salinas, J., Park, J.O. and Sivasithamparam, K. (2005).** Colonisation of seminal roots of wheat and barley by egg-parasitic nematophagous fungi and their effects on *Gaeumannomyces graminis* var. tritici and development of root rot. *Soil Biol Biochem.* 37(7). 1229–1235.

**Muthulakshmi, M., Kumar, S., Subramanian, S. and Anita, B. (2012).** Compatibility of *Pochonia chlamydosporia* with other biocontrol agents and Carbofuran. *J. Biopest.* 5. 243–245.

**Pattison, T. (2007).** Tomato root knot nematode: biology, and control. Department of Primary Industry and Fishries. Queensland, Australia.pp.50

**Pluskota, A., Horzowski, E., Bossinger, O. and von Mikecz, A. (2009).** In *Caenorhabditis elegans* nanoparticle bio-interactions becomes transparent: silica nanoparticles induce reproductive senescence. *PloS One* 4. 1–9.

**Prabhu, S., Kumar, S., Subramanian, S. and Sundaram, S.P. (2009).** Suppressive effect of *Methylobacterium fujisawaense* against root-knot nematode, *Meloidogyne incognita*. *Indian J Nematol*. 39 (2). 165–169

**Riker, A.J. and Riker, R.S. (1936)** Introduction to research on plant diseases. John's swift Co. Inc., St. Louis, Chicago NY, p-117.

**Siddiqui, M.H., Al-Whaibi, M.H., Firoz, M., Al- Khaishany, M.Y. ( 2015).** Role of Nanoparticles in Plants. *Nanotechnology and Plant Sciences* 19–35.

**Siddiqui, Y., Ali, A. and Naidu, Y., (2014).** Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants. *Crop Prot*. 65, 216-220.

**Sikora, R.A. and Fernandez, E. (2005).** Nematode parasites of vegetables. In: Luc M, Sikora RA and Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CABI publishing, pp. 319-392.

**Silva, R.V., Oliveria, R.D.L., Nascimento, K.J.T and Rodrigues, F.A .(2010).** Biochemical responses of Coffee resistance against *Meloidogyne exigua* mediated by silicon. *Plant Pathol*. 59. 586-593.

**Singh, S., Rai, A.B. and Singh, R.K. (2011).** Bio-management of root-knot disease of Chilli (*Capsicum annum*) caused by *Meloidogyne incognita*. *Int. J. Veg. Sci.* 38. 63–67.

**Singh, V., Mawar, R. and Lodha, S. (2012).** Combined effects of biocontrol agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin. *Phytopathol. Mediterr.* 51. 307-316.

**Sterling, G.R. (2011).** Biological control of plant-parasitic nematodes: an ecological perspective, a review of progress and opportunities for further research. In: Biological Control of Plant-Parasitic Nematodes: Building Coherence betiveen Microbial Ecology and Molecular Mechanisms (K.G. Davies, Y. Spiegel, ed). Springer, London, UK, 1-38.

**Suriyaprabha, R.G., Karunakaran, R., Yuvakkumar, V., Rajendran and Kannan, N. (2015).** Silica nanoparticles for increased silica availability in maize (*Zea mays* L) seeds under hydroponic conditions. *Curr Nanosci*, 8:902–908.

**Tahir, M., Rahmatullah, A., Aziz, T. and Ashraf, M. (2010).** Wheat genotypes differed significantly in their response to silicon nutrition under salinity stress. *J. Plant Nutr.* 33. 1658–1671. DOI: 10.1080/01904167.2010.496889

**Taylor, A.L. and Sasser, J.N. (1978).** In: Biology, Identification and control of Root-Knot nematodes (Meloidogyne species). A Coop. Publ. Dep. Plant Pathol., N.C. State Univ., and U.S. Agency Int. Dev., Raleigh, N.C.pp.111

**Thoden, T.C., Boppré, M. and Hallmann, J. (2009).** Effects of pyrrolizidine alkaloids on the performance of plant-parasitic and free-living nematodes. Pest Manag. *Sci*. 65. 823–830. doi:10.1002/ps.1764.

**Viggiano, J.R., de Freitas, L.G. and Lopes, E.A. (2014).** Use of *Pochonia chlamydosporia* to control of *Meloidogyne javanica* in cucumber. *Biol. control* 69. 72–77.

**Wainwright, M. (1997).** The neglected microbiology of silicon – from the origin of life to an explanation for what Henry Charlton Bastian saw. – Society of General Microbiology Quarterly, 24(3):83–85.

**Wainwright, M., Al-Wajeeh, K., Wickramansighe, N.C. and Narlikar, J.V. (2003).** Did silicon aid in the establishment of first bacterium. *Int. J. Astrobiol.* 2. 227-229.

**Wang, K., Riggs, R.D., Crippen, D. (2005)** Isolation, selection, and efficacy of *Pochonia chlamydosporia* for control of *Rotylenchulus reniformis* on cotton. *Phytopathology* 95. 890–893.

**Wasternack, C. and Hause, B. (2013).** Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 Review in Annals of Botany. *Ann. Bot.* 111. 1021–1058.

**Wesemael., W, Viaene, N. and Moens, M. (2011).** Root-knot nematodes (*Meloidogyne spp*.) in Europe. *Nematology* 13. 3–16. doi:10.1163/1388554 10X526831

**Williamson, V.M. and Hussey, R.S. (1996).** Nematode pathogenesis and resistance in plants. *Plant Cell* 8. 1735-1745.

**Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong, M.H. (2005).** Effects of biofertilizers containing N-fixer, P and K solublizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* 125. 155-166.

**Wuyts, N., Lognay, G., Swennen, R. and De waele, D. (2006).** Nematode infection and reproduction in transgenic and mutant Arabidopsis and tobacco with an altered phenylpropanoid metabolism. *J. Exp. bot.* 57. 2825-2835.

**Xie, Y., Li, B., Zhang, Q. and Zhang C. (2012).** Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of *Indocalamus barbatus* McClure. – Journal of Nanjing Forestry University (Natural Science Edition). 2. 59–63.

**Xu, X.M., Jeffries, P., Pautasso, M. and Jeger, M.J. (2011).** Combined use of biocontrol agents to manage plant diseases in theory and practice. *Phytopathology* 101.1024–1031

**Yang, J.I., Loffredo, A., Borneman, J. and Becker, J.O. (2012).** Biocontrol efficacy among strains of *Pochonia chlamydosporia* obtained from a root-knot nematode suppressive soil. *J Nematol.* 44: 67–71.

**Yang, Z., Chen, J., Dou, R., Gao, X., Mao, C. and Wang, L. (2015).** Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.). *Int. J. Environ. Res. Public Health.* 12. 15100−15109.

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