Acta Phytopathologica et Entomologica Hungarica 51 (1), pp. 43–56 (2016)
DOI: 10.1556/038.51.2016.1.4

Management of *Meloidogyne incognita* and *Macrophomina phaseolina* by Graphene Oxide on *Lens culinaris*

J. A. GUROO[1], M. KHAN[1], A. AHMAD[2], A. AZAM[2] and Z. A. SIDDIQUI[1*]

[1]Department of Botany, Aligarh Muslim University, Aligarh-202 002, India
[2]Department of Applied Physics, Z. H. College of Engineering and Technology, Aligarh Muslim University, Aligarh-202 002, India

(Received: 14 September 2015; accepted 2 December 2015)

Effect of Graphene oxide (GO) was observed on *Meloidogyne incognita* and *Macrophomina phaseolina* and on the growth of lentil in pot experiment. Treatment of plants with 10 ml solution of GO with 125, 250 and 500 ppm concentration caused a significant increase in plant dry weight over control. Inoculation of plants with *M. incognita* or *M. phaseolina* caused a significant reduction in plant dry weight over uninoculated control. Treatment of plants with 125, 250 and 500 ppm GO and subsequent inoculation with *M. incognita* or *M. phaseolina* caused a significant increase in plant dry weight over plants inoculated without GO pretreatment. Treatment of 500 ppm GO caused a greater increase in plant dry weight of *M. incognita* or *M. phaseolina* inoculated plants followed by 250 ppm and 125 ppm. Numbers of nodules per root system were high in plants without pathogen. Inoculation of *M. incognita* or *M. phaseolina* caused reduction in nodulation. However, treatment of GO in all the three concentrations had no significant effect on nodulation in plants both with and without pathogens. Treatment of GO resulted in reduced galling, nematode multiplication and root-rot index. Greater reduction in galling, nematode multiplication and root-rot index were observed in plants treated with 500 ppm GO followed by 250 ppm and 125 ppm. Indices were reduced to 4, 3 and 2, respectively, when plants with *M. phaseolina* were treated with 125, 250 and 500 ppm GO. This study shows that the use of GO is useful for the management of *M. incognita* and *M. phaseolina* on lentil.

Keywords: GO, lentil, nanoparticles, root-knot, root-rot, galling.

Lentil (*Lens culinaris* Medik.) is a bushy and annual shrub. It is popular for its lens shaped seeds, which are consumed as food in stew or other forms all over the world. The plant has many branches and pods containing one to two seeds. Lentil is one of the best and cheapest sources of vegetable proteins (Adsule et al., 1989). The enrichment of lentils with high protein makes it the preferred pulse crop to rural poor households in the world. The lentils are an important source of essential amino acids, fatty acids and trace minerals (Haq et al., 2011). Unfortunately, there are some constraints of pests and pathogens in the successful cultivation of lentil. Root-rot (*Macrophomina phaseolina*) and root-knot (*Meloidogyne incognita*) diseases cause considerable losses to this important crop.

* Corresponding author; e-mail: zaki_63@yahoo.co.in

Root nodulation is a complex symbiotic process between host plant and *Rhizobium* sp. *Rhizobium* Frank is a genus of gram negative soil bacteria that fix nitrogen. It forms a symbiotic nitrogen fixing association with roots of legumes. The association of rhizobia with plant pathogens in the rhizosphere is not always detrimental since it sometimes leads to stimulation of nodulation (Hussey and Barker, 1976). The presence of rhizobia in the rhizosphere may also protect the host root from damage caused by pathogens (Siddiqui and Husain, 1992; Siddiqui and Mahmood, 1995).

Nanotechnology has come into play and started affecting all aspects of life in a widespread manner including plant pathology. The subject nanotechnology deals with manufacturing, study and manipulation of matter at nano-scale (or atomic scale) in the size range of 1–100 nm which may be called as nanoparticles (Rajan, 2004). Any material when attenuated at nanometer scale exhibits new properties that are entirely different from its bulk counterpart due to small size and high surface to volume ratio. It is shown in terms of higher plasticity at high temperature; higher hardness, breaking strength and toughness at low temperature; higher chemical reactivity and surface energy; and high mobility in the body of an organism including cellular entry (Rajan, 2004).

Graphene is a carbon based material that can be viewed as a one atom thick sheet of graphite. Isolation and measurement of unique electronic properties of Graphene has been investigated intensively (Novoselov et al., 2004). Quickly after its initial discovery, Graphene was used to make electronic devices for a variety of applications (Allen et al., 2010). Because high quality sheets of Graphene is often prepared by chemical vapor deposition (CVD) (Li et al., 2009; Mattevi et al., 2011) which requires expensive equipment, many groups have looked at using GO as a solution processable alternative for the preparation of Graphene like materials (Compton et al., 2010; Dreyer et al., 2010b; Mao et al., 2012). Indeed, GO can be reduced in solution and as a thin film using a variety of reducing conditions, and reduction converts the GO into a material that has a large enhancement in electrical conductivity (Dreyer et al. 2010b; Gilje et al., 2007). In addition to its use in making reduced GO for electronic devices, GO has been used in catalytic oxidation (Dreyer et al., 2010a; Yeh et al., 2010; Pyun 2011) biotechnology (Guo et al., 2011; Song et al., 2011; Wang et al., 2011; Sanchez et al., 2012) and as a surfactant (Kim et al., 2010). Graphene is also related to carbon nanomaterials such as carbon nanotubes and fullerene.

During the course of survey of lentil fields of Aligarh district of U.P., we found frequent occurrence of *Macrophomina phaseolina* (Tassi) Goid and *Meloidogyne incognita* (Kofoid and White) Chitwood. Plants infected with *M. phaseolina* showed reddish to dark brown root lesions that can develop on epicotyls and hypocotyls. Brown discoloration occurring near the soil line on the epicotyl could girdle the stem while plants infected with root knot nematode *M. incognita* were characterized by poor growth and galling in roots. These two pathogens were highly destructive representing major constraints in the successful cultivation of lentil.

In the present study an attempt was made to use nanoparticle, GO, for the management of *Macrophomina phaseolina* (Tassi) Goid and *Meloidogyne incognita* (Kofoid and White) Chitwood on lentil.

Materials and Methods

Root and soil samples were collected from lentil fields of Aligarh district, U.P., India. These samples were collected in polythene bags and stored in a refrigerator at 4 °C until processing began. The samples were examined for the presence of the root-knot nematode *Meloidogyne* spp., and root-rot fungus *Macrophomina phaseolina.*

Isolation and identification of root-knot nematode M. incognita

Root galls were dissected to take out the females of root-knot nematodes. Identification of *Meloidogyne* sp. was made on the basis of perineal patterns (Taylor and Sasser, 1978). Soil samples were also processed for the isolation of nematodes by Cobb's sieving and decanting technique followed by a Baermann funnel (Southey, 1986). Identification of root-knot juveniles were also made from the nematode suspension.

Isolation of root-rot fungus from infected lentil roots

Root showing root-rot symptoms were transferred to sterilized Petri dishes containing sterilized distilled water and gently freed of soil particles. These parts were transferred to other Petri dishes and the process was repeated until all adhering soil particles were removed. Later, these infected roots were cut into approximately 5 mm pieces and transferred to a Petri dish containing 0.1% sodium hypochlorite (NaOCl) solution. After one minute the pieces were washed at least thrice in distilled water and dried on filter paper. Five of these pieces were then plated in each of 10 Petri dishes containing PDA (Potato dextrose agar) using sterilized forceps under aseptic conditions. Petri dishes were incubated at 25 ± 2 °C for 10 days. The fungus that developed from infected roots was examined. *M. phaseolina* forms black colonies in PDA and identification was done on the basis of Ghos and Ghos (1990). Hyphal branches generally form at right angles to parent hyphae, but branching is also common at acute angles. Mycelium is septate 1.5 to 2.5 μm wide, hyaline first and turns black later. Shapes of sclerotia are mostly irregular. Sclerotia are hyaline to light brown about 90 μm in diameter under laboratory condition. On confirmation of its identity as *M. phaseolina* its pure culture was prepared. The pure culture was stored at 5 °C until used.

Preparation and characterization of GO

GO was prepared using modified Hummer's method (Hummers and Offeman, 1958) using AR grade chemicals. Briefly, 2 g of graphite powder was placed in a flask. Then 46 ml of H_2SO_4 was added with stirring in an ice bath for 15 min, 6 g of $KMnO_4$ was slowly added to avoid sudden increase in temperature over about half an hour keeping temperature below 20 °C. Stirring was continued for 35 minutes during which the colour changed from purple to purple green. After 35 min, ice-bath was removed and mixture was heated to 35 °C and maintained the temperature for 30 min, thick paste was formed.

Subsequently, 92 ml deionized (DI) water was gradually added so that the temperature increases to 98 °C and external heat was introduced to maintain temperature at 98 °C for 15 min. After 15 min, 280 ml DI water and 20 ml of hydrogen peroxide (H_2O_2) (30 wt% aqueous solution) was further added. Stirring continued for 2 h so that colour of solution turned to brilliant yellow accompanied by bubbling. Resulting solution was filtered and washed with 250 ml of 10% HCl, DI water and ethanol three times each by centrifugation until pH became neutral. Resultant mixture was sonicated by adding 20 ml of DI water and dried at 60 °C in vacuum oven for 24 h to get the GO. The crystalline structures of the GO were investigated by X-ray diffraction (XRD) using a Rigaku miniflex II powder diffractometer in 20 range of 5°–50° (Cu K_α operated at 30 kV, 15 mA). XRD pattern of GO and graphene has been shown in Fig. 1, respectively. The GO and Graphene patterns

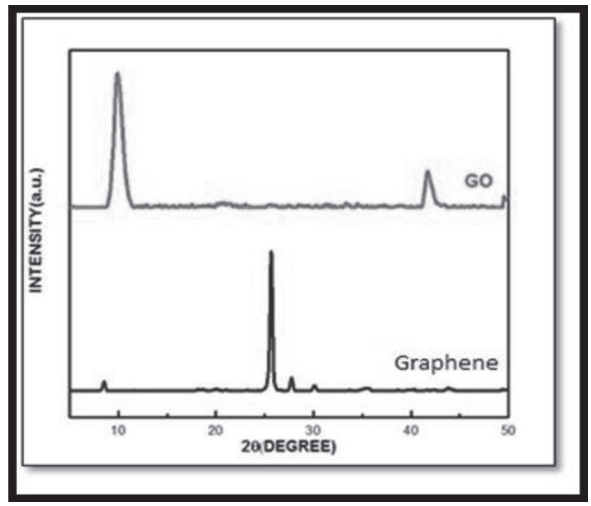
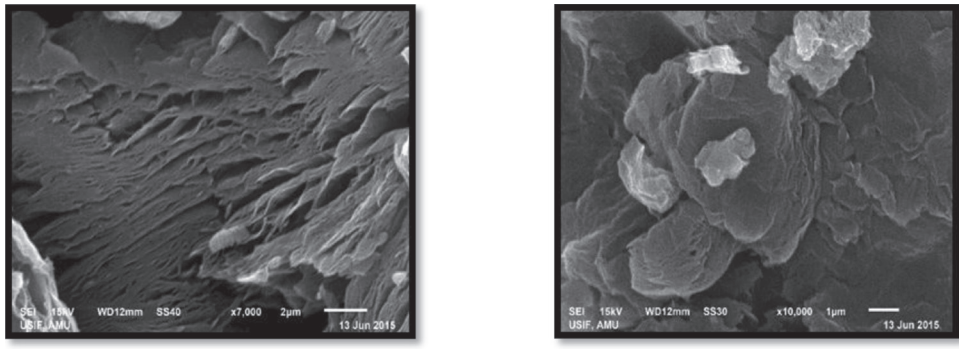


Fig. 1. XRD graph of Graphene oxide and Graphene



(a) (b)

Fig. 2. SEM images of GO with (a) × 7, 000 and (b) × 10, 000 magnification

reveal intense and sharp peaks located at 25.7° and 9.94°, respectively (Fang et al., 2009). The scanning electron microscopy (SEM) images of GO with 7,000 and 10,000 magnifications have been shown in Fig. 2 (a and b). GO consists of randomly aggregated, thin, crumpled sheets closely associated with each other to form a disordered solid. GO has layered structure, which shows ultrathin and homogeneous films.

Effect of GO on hatching and mortality of root knot nematodes

The effect of 3 concentrations of GO (125 ppm, 250 ppm and 500 ppm) was observed separately on the hatching and mortality of *Meloidogyne incognita* in small Petri dishes at 25 ± 1 °C. Twenty egg masses of almost similar size were handpicked with sterilized forceps from the roots of eggplant (*Solanum melongena* L.) and were placed for hatching in 20 ml suspension. Twenty ml suspensions were made with 19 ml distilled water and 1 ml GO of 125 ppm / 250 ppm / 500 ppm. For control, twenty egg masses were placed in 20 ml double distilled water. Each set was replicated five times and numbers of juveniles hatched were counted under stereomicroscope. Experiment was repeated and pooled data were presented (Table 1).

Effect of GO on the root-rot fungus

The antifungal activity of GO was determined by adding 1 ml GO of 125 / 250 / 500 ppm separately into 100 ml autoclaved PDA just before pouring in Petri dishes. *Macrophomina phaseolina* was inoculated on the plates containing different concentration of GO in PDA. Each set was replicated five times and experiment was repeated. The dishes were incubated at 25 ± 1 °C and observed every 24 h up to 10 days. Antifungal activity of GO was determined by measuring the growth of colonies of fungus in control and different concentration 10 days after inoculation. Percent inhibition was calculated by comparing to a control (Table 1).

Table 1

Effects of 3 concentrations of Graphene oxide (GO) on the hatching and mortality of *M. incognita* and activity against *M. phaseolina*

Treatments	No. of *M. incognita* J2 hatched	Mortality of *M. incognita* J2	% inhibition in *M. incognita* J2 hatching	% Mortality of *M. incognita* J2	Activity against *M. phaseolina* %
Double Distilled Water	262a	12d	–	4.6	–
Graphene oxide (GO) 125 ppm	141b	72b	46.2	51.1	26.4
GO 250 ppm	98c	79a	62.6	80.6	51.3
GO 500 ppm	46d	44c	82.4	95.7	78.2

* Values within column followed by different letters are significantly different at P = 0.05 based on Duncan's multiple range test

Preparation and sterilization of soil mixture

Sandy loam soil is collected from a field belonging to the Department of Botany, AMU, Aligarh and passed through a 10 mesh sieve. The soil, river sand and organic manure were mixed in a ratio 3:1:1 and 9 cm diameter clay pots were each filled with 1 kg of the mixture. A little water was poured into each pot to just wet the soil surface before sterilization at 137.9 kPa for 20 minutes. Sterilized pots were allowed to cool at room temperature before use.

Raising and maintenance of test plants

Lentil (*Lens culinaris* Medik.) seeds of cultivar Malika were surface sterilized with 0.1% sodium hypochlorite (NaOCl) for 2 minutes and rinsed three times with sterile water. Three seeds were sown in steam sterilized soil in 15 cm diameter clay pots and one week after germination thinning was done to maintain single seedling per pot. Seedlings were subjected to the treatments listed in Table 2. Un-inoculated plants served as a control and plants were kept in a glass house at 20 ± 2 °C. Pots were arranged in a randomized block design and each treatment was replicated 5 times. Pots were watered as needed and experiment was terminated 90 days after inoculation. Experiment was repeated once. Data of both the experiments were similar and means of both the year data have been presented.

Fungus inoculum

For obtaining sufficient inoculum, *Macrophomina phaseolina* was cultured on Richard's liquid medium having following composition:

Potassium nitrate	10.0 g
Potassium dihydrogen phosphate	5.0 g
Magnesium sulphate	2.5 g
Ferric chloride	0.02 g
Sucrose	50.0 g
Distilled water	1000 ml

The medium was prepared and filtered through muslin cloth, sterilized in an autoclave at 103.4 kPa for 15 minutes in 250 ml Erlenmeyer flasks each containing 80 ml liquid medium. *Macrophomina phaseolina* was inoculated in each flask with the help of inoculation needle. Inoculated flasks were incubated at 25 ± 1 °C for about 15 days to allow sufficient growth of the fungus. Mycelia mats of the fungus were collected on blotting papers separately to absorb excess of water and nutrients. The inoculum of fungus was prepared by mixing 50 g mycelium in 500 ml distilled water and blending it for 30 seconds in Waring blender. Ten ml of this suspension containing 1 g of fungal matterial was used as inoculum. Pure culture of *M. phaseolina* was continuously maintained on PDA by re-inoculation after every 15 days.

Rhizobium inoculum

Hundred g commercial culture of *Rhizobium* sp. (lentil strain) was suspended in 1000 ml distilled water and 10 ml (equivalent to 1 g inoculum) was inoculated in each pot around the seeds at the time of sowing.

Preparation of nematode inoculum

Meloidogyne incognita was collected from the lentil roots and multiplied on the roots of egg plants (*Solanum melongena* L.) using single egg mass. Large numbers of egg masses from heavily infected eggplant roots were hand-picked with the help of sterilized forceps from the previously maintained pure culture of *M. incognita*. The egg masses were washed with distilled water and placed in a small sieve (9 cm diameter with 1-mm pore size) containing crossed layers of tissue paper. The sieve was placed in a Petri dish containing distilled water deep enough to contact the egg masses. A number of these assemblies were kept in an incubator running at 25 ± 1 °C in order to obtain the required number of second-stage juveniles for inoculation. The hatched second-stage juveniles were collected from the Petri dishes every 24 h, fresh water was added, and the process was repeated. For counting nematode juveniles, an average of 5 counts was made to determine the density of nematodes in the suspension. The volume of nematode suspension was so adjusted that each ml may contain 200 ± 5 nematodes. Ten ml of this suspension (i.e. 2000 freshly hatched juveniles) was added to each pot around lentil seedling.

Inoculation technique

Rhizobium was inoculated in all plants at the time of sowing. Two weeks old lentil plants were used for inoculation. For inoculation of *M. phaseolina, M. incognita*, and GO soil around the roots was carefully removed aside without damaging the roots. The inoculum suspensions were poured around the roots and the soil was replaced. In control treatment, water was added in equal volume to the suspension. The treatments applied are shown in Table 2. There were 4 treatments comprising of (1) Control; (2) GO 125 ppm (A); (3) GO 250 ppm (F); (4) GO 500 ppm. These 4 treatments were tested with (I) Control; (II) *M. phaseolina;* (III) *M. incognita* ($4 \times 3 = 12$). Each treatment was replicated 5 times ($12 \times 5 = 60$ pots). Experiment was repeated and pooled data were presented.

Evaluation of plant growth, disease advancement and symptoms

The plants were harvested 90 days after inoculation. Data on plant length, plant fresh weight, numbers of nodules and galls and root rot index were recorded. The length of plant was recorded in centimeter from the top of the first leaf to the end of the root. Excess water was removed by blotting before weighing the plant. The plants were cut with

Table 2

Effect of Graphene oxide (GO) nanoparticles of root-knot nematode *Meloidogyne incognita* and root-rot fungus *Macrophomina phaseolina* on lentil

Treatments		Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	% increase in plant dry weight	No. of nodules per root system	No. of galls per root system	Root–rot index
Control	Control	41.9cd	6.77de	1.72de	–	17a	–	–
	GO125 ppm	43.2bc	7.24c	2.04c	18.60	18a	–	–
	GO250 ppm	44.6b	7.92b	2.35b	36.63	16a	–	–
	GO500 ppm	46.3a	8.57a	2.69a	56.40	17a	–	–
M. incognita	Control	33.5g	4.82g	0.98h	–	7b	35a	–
	GO125 ppm	37.2ef	5.64f	1.26gh	28.57	8b	23b	–
	GO250 ppm	40.8d	6.50de	1.57ef	60.20	6b	16c	–
	GO500 ppm	42.6c	7.49bc	1.91cd	94.90	9b	09d	–
M. phaseolina	Control	33.9g	4.90g	1.02h	–	8b	–	5
	GO125 ppm	36.2f	5.24fg	1.35fg	32.35	7b	–	4
	GO250 ppm	38.9e	6.39e	1.58ef	54.90	8b	–	3
	GO500 ppm	42.1cd	7.04cd	1.96cd	92.11	8b	–	2

GO* = Graphene oxide

* Values within column followed by different letters are significantly different at P = 0.05 based on Duncan's multiple range test

knife above the base of the root emergence zone to separate shoot and root. Shoots and roots were kept in envelope at 60 °C for 4 days before weighing for dry weight determination. A 250 g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting technique followed by Baermann funnel extraction (Southey, 1986). Nematode suspension was collected after 24 h and the numbers of nematodes were counted in five aliquots of 1 ml of suspension from each sample. The means of five counts were used to calculate the population of nematodes per kg soil. To estimate the number of juveniles, eggs and females inside the roots, 1 g subsample of roots was macerated in a Waring blender and counts were made from the suspension thus obtained. Numbers of nematodes present in roots were calculated by multiplying the number of nematodes present in 1 g of root by the total weight of root. Root rot index was determined by scoring the severity of disease on a scale ranging from 0 (no disease) to 5 (severe root-rot).

Statistical analysis

The data were analyzed statistically (Dospekhov, 1984). Least significant differences (L.S.D.) were calculated at P = 0.05. Duncan's multiple range test (DMRT) was applied to denote the significant differences between the treatments. Figures 3 and 4 error bars represent Standard error.

Results

Effects of GO on hatching and mortality of M. incognita

Effects of 3 concentrations of GO, i.e. 125, 250, 500 ppm were studied on the hatching and mortality of *M. incognita* (Table 1). All the 3 concentrations had inhibitory effect on hatching and mortality of *M. incognita*. Highest concentration of GO, i.e. 500 ppm caused maximum inhibition of *M. incognita* hatching and mortality of J2 followed by 250 and 125 ppm concentrations. Addition of 500 ppm GO caused 82.4% reduction in hatching while 62.6 and 46.2% inhibition was observed with 250 and 125 ppm GO. Similarly, 500 ppm concentration of GO caused 95.7% mortality while 250 and 125 ppm concentrations caused 80.6 and 51.1% mortality of second stage juveniles of *M. incognita* (Table 1).

Effects of GO against root-rot fungus

Effects of three concentrations of GO, i.e. 125, 250, 500 ppm were studied on *M. phaseolina* (Table 1). All the three concentrations had inhibitory effect on the growth of *M. phaseolina*. Highest concentration of GO, i.e. 500 ppm caused maximum inhibition in the growth of *M. phaseolina* followed by 250 ppm and 125 ppm concentration. Addition of 125 ppm GO caused 26.4% inhibition in fungus growth while 51.3 and 78.2% was caused by 250 ppm and 500 ppm GO (Table 1).

Pot experiment

Treatment of plants with 10 ml solution of GO in 3 concentrations, i.e. 125, 250 and 500 ppm caused a significant increase in plant dry weight over control (Table 2). Treatment of 500 ppm concentration of GO caused a greater increase in plant dry weight followed by 250 ppm and 125 ppm concentrations. Treatment of 125 ppm GO caused 18.60% increase in plant dry weight over uninoculated control while application of 250 ppm and 500 ppm GO caused 36.63% and 56.40% increase in plant dry weight over uninoculated control (Table 2).

Inoculation of plants with *M. incognita* caused a significant reduction in plant dry weight over uninoculated control (Table 2; Fig. 3). Treatment of plants with 125, 250 and 500 ppm GO before inoculation with *M. incognita* caused a significant increase in plant dry weight over plants inoculated with *M. incognita* alone. Treatment of 500 ppm GO caused a greater increase in plant dry weight of *M. incognita* inoculated plants followed by 250 ppm and 125 ppm. Treatment of 125 ppm GO before inoculation with *M. incognita* caused 28.57% increase in plant dry weight over plants with nematode alone while application of 250 ppm and 500 ppm GO to plants with *M. incognita* caused 60.20% and 94.90% increase in plant dry weight (Table 2; Fig. 3).

Inoculation of *M. phaseolina* caused a significant reduction in plant dry weight over uninoculated control (Table 2; Fig. 3). Treatment of 125, 250 and 500 ppm GO before inoculation with *M. phaseolina* caused significant increases in plant dry weight over plants inoculated with *M. phaseolina* alone. Treatment of 500 ppm GO caused a greater

increase in plant dry weight of *M. phaseolina* inoculated plants followed by 250 ppm and 125 ppm. Treatment of 125 ppm GO before inoculation with *M. phaseolina* caused 32.35% increase in plant dry weight over plants with *M. phaseolina* alone while application of 250 ppm and 500 ppm GO to *M. phaseolina* inoculated plants caused 54.90 and 92.11% increase in plant dry weight (Table 2; Fig. 3).

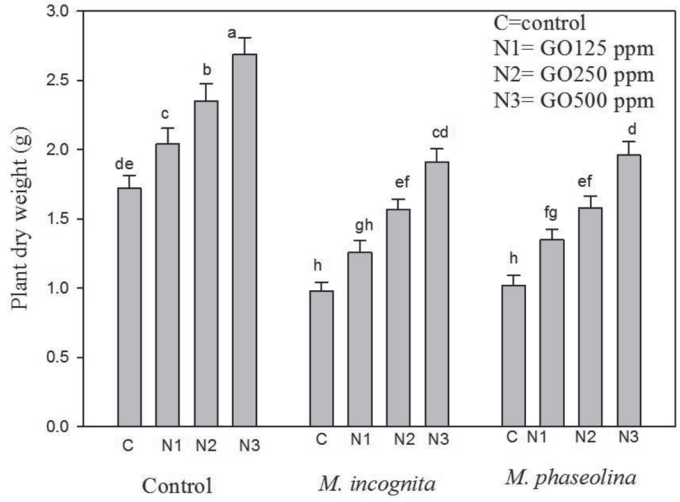


Fig. 3. Effect of Graphene oxide nanoparticles on the plant dry weight of lentil in *Meloidogyne incognita* and *Macrophomina phaseolina* inoculated plants. Error bars represent standard error. Different alphabets represent significant difference between the treatments at P = 0.05

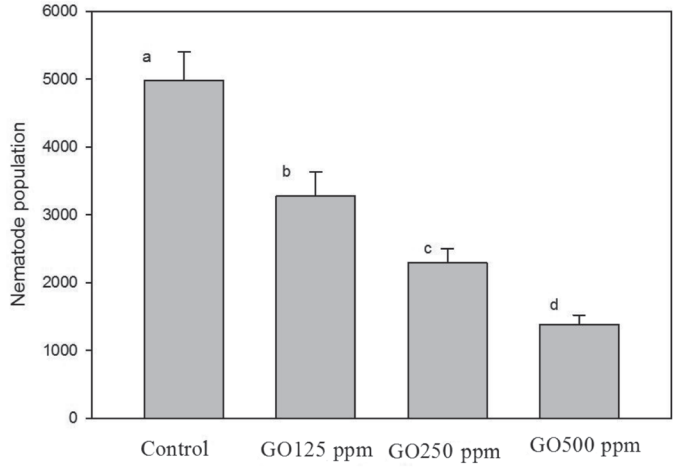


Fig. 4. Effect of Graphene oxide nanoparticles on population of *Meloidogyne incognita* on lentil. Error bars represent standard error. Different alphabets represent significant difference between the treatments at P = 0.05

Numbers of nodules per root system were high in plants without *M. incognita* or *M. phaseolina* (Table 2). Inoculation of *M. incognita* or *M. phaseolina* caused reduction in nodulation over plants without pathogens. Inoculation of *M. incognita* caused over all 55.88% reduction in nodulation while inoculation of *M. phaseolina* caused about 45.59% reduction in nodulation. Treatment of GO in three concentrations (125, 250 and 500 ppm) had no significant effect on nodulation in plants both with or without pathogens (Table 2).

Root galling and nematode multiplication was high in plants inoculated with *M. incognita* alone (Table 2; Fig. 4). Treatment of GO in all the three concentrations (125, 250 and 500 ppm) reduced galling and nematode multiplication. Greater reduction in galling and nematode multiplication was observed in plants treated with 500 ppm GO followed by 250 ppm and 125 ppm. Treatment of 125, 250 and 500 ppm caused 34.3%, 54.3% and 74.3% reductions in galling, respectively, while treatment of 125, 250 and 500 ppm resulted in 34.9, 54.0 and 72.3% reduction in nematode multiplication (Table 2; Fig. 4).

Root-rot index was found 5 when *M. phaseolina* was inoculated alone (Table 2). Treatment of GO in all the 3 concentrations, i.e. 500, 250 and 125 ppm reduced root rot index of *M. phaseolina* inoculated plants. Root-rot index was reduced to 4 when 125 ppm GO was applied to *M. phaseolina* inoculated plants. Indices were 3 and 2, respectively when plants with *M. phaseolina* were treated with 250 and 500 ppm GO (Table 2).

Discussion

Application of GO in all the three concentrations inhibited the growth of root-rot fungus *M. phaseolina* and also had adverse effect on hatching and mortality of root knot nematode *M. incognita.* The strong antifungal and antibacterial properties of GO have been reported by Sawangphruk et al. (2012); Wang et al. (2013, 2014). The addition of GO in culture medium not only inhibited the growth of *M. phaseolina* but cell wall of the mycelium was disrupted. The inhibition of growth and disruption of cell wall of fungus was increased with increase in the concentration of GO. Cell membrane disruption of fungal spores was verified by examining the leakage of electrolytes from *F. graminearum* and *F. oxysporum* by Chen et al. (2014). Their results indicated that about 57.7% of the total electrolytes leaked out of the *F. graminearum* cells and 53.6% out of *F. oxysporum* even 300 min after exposure to 500 mg mL^{-1} GO, suggesting that GO could indeed disrupt the phospholipids of fungal membranes (Chen et al., 2014). Injury was manifested as an increased leakage of electrolyte. The disruption of membrane has a large impact on membrane potential changes and membrane-associated energy-transducing system, such as intra- and extra-cellular ATP pools (Lyon and Alvarez, 2008). The activation of membrane potential is associated with spore germination. One of the earliest events in the germination of spores is the activation of membrane potential (Harris, 2005). Thus, the perturbation of membrane integrity and the subsequent leakage of electrolytes may have relationship with inactivation and growth of fungus. The antimicrobial activity of GO, is associated with its facile surface modification, high mechanical strength, good water dispersibility, and photoluminescence (Singh et al., 2011). In addition, antifungal mechanism

of GO deduced to target the spores by depositing on the surface of spores, inhibiting water uptake and inducing plasmolysis (Wang et al., 2014).

GO also had adverse effect on the hatching and mortality of *M. incognita*. It is possible that toxicity of GO inhibited the hatching of *M. incognita*. Hatched 2nd stage juveniles of *M. incognita* showed mortality within 6 hours of hatching. Observation of dead 2nd stage juveniles under microscope showed their deformed shape. It is possible that the leakage of electrolytes from body wall of nematodes may have resulted in the mortality of nematodes.

In pot experiment, application of GO resulted in the increased growth of both pathogen inoculated and uninoculated plants. Khodakovskaya et al. (2012) reported the application of carbon nanotubes as regulators of seed germination and plant growth. Multiwalled carbon nanotubes (MWCNTs) have the ability to enhance the growth of tobacco cell culture by 55–64% when compared to control at a wide range of concentrations from 5–500 μg/ml (Khodakovskaya et al., 2012). Carbon nanotubes (CNTs) can regulate cell division and plant growth by a unique molecular mechanism that is related to the activation of water channels (aquaporins) and major gene regulators of cell division and extension (Khodakovskaya et al., 2012). Therefore, increase in plant growth in the absence of plant pathogens with increase in GO concentration can be attributed to role of carbon nanotubes as regulators of seed germination and plant growth (Khodakovskaya et al., 2012).

Increase in growth of *M. incognita* and *M. phaseolina* inoculated plants was also observed. Adverse effect of GO was observed in our laboratory test on the growth of *M. phaseolina* and hatching and mortality of *M. incognita.* Widely recognized antimicrobial mechanisms of GO include the synergistic effects of membrane disruption of the cell envelope and oxidative stress, which are related to the physical and chemical properties of GO (Akhavan and Ghaderi, 2010; Liu et al., 2011). GO concentration dependent inhibitory effects on a phytopathogenic fungus were observed not only in this study but by others as well (Chen et al., 2014). Present study reveals that GO inhibited both pathogens, i.e. *M. incognita* and *M. phaseolina*, which is evident by reduced root galling and nematode multiplication and reduced root-rot index which resulted in increased growth of pathogen inoculated plants.

The above results indicate that GO can display broad spectrum antimicrobial activity towards phytopathogens and this antimicrobial activity is concentration-dependent. Therefore, the use of GO can be expected to serve as a long-term effective measure to control pathogenic microbes, in contrast to some chemical pesticides.

Literature

Adsule, R. N., Kadam, S. S. and Leung, H. K. (1989): Lentil. In: D. K. Salunkhe and S. S. Kadam (eds): Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization. Vol. 11, Boca Raton, FL: CRC Press, 131 p.

Akhavan, O. and Ghaderi, E. (2010): Toxicity of graphene and GO nanowalls against bacteria. ACS Nano 4, 5731–5736.

Allen, M. J., Tung, V. C. and Kaner, R. B. (2010): Honeycomb carbon: a review of graphene. Chem. Rev., 110, 132.

Chen, J., Peng, H., Wang, X., Shao, F., Yuan, Z. and Han, H. (2014): GO exhibits broad-spectrum antimicrobial activity against bacterial phytopathogens and fungal conidia by intertwining and membrane perturbation. Nanoscale 6, 1879–1889.

Compton, O. C. and Nguyen, S. T. (2010): GO, Highly reduced GO, and graphene: Versatile building blocks for carbon-based materials. Small 6, 711.

Dospekhov, B. A. (1984): Field Experimentation. Statistical Procedures. Mir Publishers, Moscow, Russia, pp. 240–243.

Dreyer, D. R., Jia, H-P. and Bielawski, C. W. (2010a): GO: a convenient carbocatalyst for facilitating oxidation and hydration reactions. Angew. Chem. Int. Ed. 49, 6813–6816.

Dreyer, D. R., Park, S., Bielawski, C. W. and Ruoff, R. S. (2010b): The chemistry of GO. Chem. Soc. Rev., 39, 228.

Fang, M., Wang, K., Lu, H., Yang, Y. and Nutt, S. (2009): Covalent polymer functionalization of graphene nanosheets and mechanical properties of composites. Royal Soc. Chem. 19, 7098–7105.

Ghosh, I. and Ghosh, T. (1990): Pycnidia and sclerotia of *Macrophomina phaseolina* (Tassi) Goid on jute and identity of the sclerotial isolates. J. Mycopathol. Res. 28, 111–120.

Gilje, S., Han, W., Wang, M., Wang, K. L. and Kaner, R. B. (2007): A Chemical route to graphene for device applications. Nano Lett, 7, 3394.

Guo, Y. J., Deng, L., Li, J., Guo, S. J., Wang, E. K. and Dong, S. J. (2011): Hemin–graphene hybrid nanosheets with intrinsic peroxidase-like activity for label-free colorimetric detection of single nucleotide polymorphism. ACS Nano 5, 1282–1290.

Haq, M. Z., Ahmad, S., Shad, M. A., Iqbal, S., Qayum, M., Ahmad, A., Luthria, D. L. and Amarowicz, R. (2011): Compositional studies of lentil (*Lens culinaris* Medik.) cultivars commonly grown in Pakistan. Pak. J. Bot., 43, 1563–1567.

Harris, S. D. (2005): Morphogenesis in germinating *Fusarium graminearum* macroconidia. Mycologia 97, 880–887.

Hummers, W. S. and Offeman, R. E. (1958): Preparation of graphitic oxide. J. Am. Chem. Soc. 80, 1339.

Hussey, R. S. and Barker, K. R. (1976): Influence of nematodes and light sources on growth and nodulation of soybeans. J. Nematol. 8, 48–52.

Khodakovskaya, M. V., de Silva, K., Biris, A. S., Dervishi, E. and Villagarcia, H. (2012): Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano, 6, 2128–2135.

Kim, J., Cote, L. J., Franklin Kim, F., Yuan, W., Shull, K. R. and Huang, J. J. (2010): GO sheets at interfaces. Am. Chem. Soc. 132, 8180–8186.

Li, X., Cai, W., An, J., Kim, S., Nah, J., Yang, D., Piner, R., Velamakanni, A., Jung, I., Tutuc, E., Banerjee, S. K., Colombo, L. and Ruoff, R. R. (2009): Large-area synthesis of high-quality and uniform graphene films on copper foils. Science 324, 1312.

Liu, S. B., Zeng, T. H., Hofmann, M., Burcombe, E., Wei, J., Jiang, R. R., Kong, J. and Chen, Y. (2011): Antibacterial activity of graphite, graphite oxide, GO, and reduced GO: membrane and oxidative stress. ACS Nano 5, 6971–6980.

Lyon, D. Y. and Alvarez, P. J. J. (2008): Fullerene water suspension (nC60) exerts antibacterial effects via ROS-independent protein oxidation. Environ. Sci. Technol. 42, 8127–8132.

Mao, S., Pu, H. and Chen, J. (2012): GO and its reduction: Modeling and experimental progress. RSC Adv. 2, 2643.

Mattevi, C., Kima, H. and Chhowalla, M. J. (2011): A review of chemical vapour deposition of graphene on copper. Mater. Chem. 21, 3324.

Novoselov, K. S., Geim, A. K., Morozov, S. V., Jiang, D., Zhang, Y., Dubonos, S. V., Grigorieva, I. V. and Firsov, A. A. (2004): Electric field effect in atomically thin carbon films. Science 306, 666–669.

Pyun, J. (2011): GO as catalyst: Application of carbon materials beyond nanotechnology. Angew. Chem. Int. 50, 46.

Rajan, M. S. (2004): Nano: The Next Revolution. National Book Trust, 81-237-4305-X (620.5 T96N E06700), New Delhi, India, pp. 1–179.

Sanchez, V. C., Jachak, A., Hurt, R. H. and Kane, A. B. (2012): Biological interactions of graphene-family nanomaterials: an interdisciplinary review. Chem. Res. Toxicol. 25, 15–34.

Sawangphruk, M., Srimuk, P., Chiochan, P., Sangsri, T. and Siwayaprahm, P. (2012): Synthesis and antifungal activity of reduced GO nanosheets. Carbon 50, 5156–5161.

Shahriary, L. and Athawale, A. A. (2014): Graphene oxide synthesized by using modified hummers approach. Intern. J. Renew. Energy Environ. Engineer. 2, 58–63.

Siddiqui, Z. A. and Husain, S. I. (1992): Interaction between *Meloidogyne incognita* race 3, *Macrophomina phaseolina* and *Bradyrhizobium* sp. in the root-rot disease complex of chickpea *Cicer arietinum*. Fundam. Appl. Nematol. 15, 491–494.

Siddiqui, Z. A. and Mahmood, I. (1995): Role of plant symbionts in nematode management: A review. Bioresource Technol. 54, 217–226.

Singh, V., Joung, D., Zhai, L., Das, S., Khondaker, S. I. and Seal, S. (2011): Graphene based materials: past, present and future. Prog. Mater Sci. 56, 1178–1271.

Song, Y. J., Chen, Y., Feng, L. Y., Ren, J. S. and Qu, X. G. (2011): Selective and quantitative cancer cell detection using target-directed functionalized graphene and its synergetic peroxidase-like activity. Chem. Commun. 47, 4436–4438.

Southey J. F. (1986): Laboratory Methods for Work with Plant and Soil Nematodes. Ministry of Agriculture, Fisheries and Food Reference Book No. 402, 202 p.

Taylor, A. L. and Sasser, J. N. (1978): Biology, Identification and Control of Root-knot Nematodes (*Meloidogyne* spp.). North Carolina State University, Raleigh, Graphics, 111 p.

Wang, X., Liu, X., Chen, J., Han, H. and Yuan, Z. (2014): Evaluation and mechanism of antifungal effects of carbon nanomaterials in controlling plant fungal pathogen. Carbon 68, 798–806.

Wang, X., Liu, X. and Han, H. (2013): Evaluation of antibacterial effects of carbon nanomaterials against copper-resistant *Ralstonia solanacearum*. Colloids and Surfaces B: Biointerfaces 103, 136–142.

Wang, Y., Li, Z., Wang, J., Li, J. and Lin, Y. (2011): Graphene and GO: biofunctionalization and applications in biotechnology. Trends Biotechnol. 29, 205.

Yeh, T-F., Syu, J-M., Cheng, C., Chang, T-H. and Teng, H. (2010): Graphite oxide as a photocatalyst for hydrogen production from water. Adv. Funct. Mater 20, 2255–2262.