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# EFFECT OF HOMEOPATHIC NEMATICIDE PELLETS ON PLANT-NEMATODE INTERACTION UNDER CONTROLLED CONDITIONS

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**Abstract**

An experiment was conducted at Department of Botany, University of Karachi to explore the efficacy of homeopathic drug pellets for the control of root knot nematode and improved plant growth of crops. Homeopathic drug pellets of *Santonine*-43 and *Kent*-20 used at 75% concentration, respectively, showed excellent results in the control of *M. javanica* infection but also improved the plant height, weight and yield of all tested crops such as mung bean, mash bean, okra and sunflower followed by 50% concentration in contrast to control. However, 75% concentration of *Kent*-20 pellets were found best in reducing the galls formation produced by root knot nematodes, while *Santonine*-43 pellets were best in the improvement of growth of tested crop plants.

**Key words:** Homeopathic drugs, Nematode control and Pellets preparation.

## Introduction

Plant nematodes have been identified as hidden enemies, causing huge losses in crops (Savary *et al*., 2006; Montesinos, 2007).Root Knot Nematodes (RKNs) especially *Meloidogyne* spp., are reasonably the most important with respect to agricultural productivity in quality limiting pathogens occurs worldwide (Javed *et al*., 2006). Disease incidence in Pakistan in which losses result from the nematodes estimates between 5-25% (Maqbool, 1988) and recently recorded between 75-100% (Khan, 2009). Of the total, seventy *Meloidogyne* spp., are being identified so far (Luc *et al*., 1988) but only four species namely, *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood are the most copious and harmful nematode in Pakistan which infects about more than hundred plant species (Maqbool & Shahina, 2001). *Meloidogyne javanica*, one of the four most common species of 'Plant Parasitic Nematodes' (PPNs), is extremely polyphagous that attacks both monocotyledons and dicotyledons crops (Perry *et al*., 2009). Nematode produced infection on the second-stage juvenile ($J_2$) which penetrates into the plant through the roots by the oesophageal glands which developed the feeding structure and migrates into the actively dividing plants cells producing hypertrophy and hyperplasia giving the characteristic of galls commonly known as 'root knots' (Srivastava, 1973; Gheysen & Fenoll, 2002; Williamson & Kumar, 2006). Due to the formation of galls produced by root knot nematodes it brings various changes in physiology and morphology of the plants (Williamson & Gleason, 2003). The changes that occur within the roots includes; disturbance of the root xylem, interferes in the nodulation and nitrogen fixation production, epidermal and cortical tissues which in turn affect water and nutrient uptake result in wilting, stunted growth and reduced light interception which results in decrease of photosynthetic efficiency (Kirkpatrick *et al*., 1991; Agrios, 2005).

Fastest way of nematode management is completely based on the nematicides due to high population growth rate, which demands increase in crop production (Hallmann *et al*., 2009). Majority of nematicides are hazardous to the environment because they leave high toxic residual effect and disturb the ecological balance (Chitwood, 2002). Prolonged and overuse leads to development of chemical resistance in root knot nematodes, therefore the public prefer environmentally safe, reasonably priced, less time consuming and agronomically useful (Serfoji *et al*., 2010). Due to this demerit, there is an urgent need for an eco-friendly substitute for nematode management (Siddiqui & Alam, 1985). Many researchers recommended various strategies for controlling pathogenic nematodes which includes; use of resistant cultivars, incorporation of organic matter, use of antagonistic micro-organism or crop rotation with different host plants (Oliveira *et al*., 2005; Naserinasab *et al*., 2011; Soltani *et al*., 2013; Tavares-Silva *et al*., 2015). These eco- friendly methods have been adopted as application of nematicides are banned due to health hazards and environmental concerns due to its toxicity effect, although nematicides play an important role in crop protection when other practical and economical measures are lacking (Mukhtar & Pervaz, 2003).

Of the various approaches that could be adopted for the control of nematodes, use of homeopathic drugs has recently developed interest among nematologists which is found environment friendly and reduced the population of nematodes as homoeopathy consists in prescribing substances; either highly diluted or potentized form can be applied to all living beings, including plants recently, which provide potential technology for sustainable agriculture (Rossi *et al.,* 2004; Toledo *et al*., 2009). Nowadays, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries (Singh, 2011) could offer potential benefits because homeopathic preparations, due to their extreme dilution level do not lead to any accumulation and toxicity in the environment, offers relatively cheap and have no ecological side-effects considered as harmless (Betti *et al*., 2006; Grazia *et al*., 2014).Treatment of plants through homeopathy helps to increase the resistance or tolerance levels against pathogens such as diseases caused by viruses, fungi, nematodes and bacteria, beside, it also increases the production of biomass (Carneiro *et al*., 2010). Due to these attributes, homeopathy optimally suited to the biodynamic agriculture, in which plants and their interactions with the environment applied as a unified living organism (Carpenter-Boggs *et al*., 2000;

Albrecht *et al*., 2002). Application of homeopathy to agriculture can also be aimed by improving the physiological and qualitative characteristics of plants (Marques *et al*., 2006) in addition to their resistance tobiotic (insects and pathogens) and abiotic (physical andchemical changes) stress (Scofield, 1984; Shah-Rossi *et al*., 2009). Used of homeopathic drugs in the control of *Meloidogyne* spp., has been proposed by various researchers which shows nematicidal effect and economically adequate approach for pathogen control (Datta, 2006; Sukul *et al*., 2006; Carneiro, 2011). An attempt was made during this research to control the plant parasitic nematode, *M. javanica* under field conditions and to improve the growth and yield of crop plants.

## Materials and Methods

**Pellets preparation with homeopathic drugs:** Two homeopathic drugs such as, *Kent*-20 and *Santonine*-43 were purchased from the medicinal market of Karachi, Pakistan. Slight modification of preparation of pellets was made on the method given by Hanif *et al*., (2015). *Kent*-20 and *Santonine*-43 with the concentrations of 75 and 50% v/w were mixed with pyrophyllite (hydrous aluminum silicate-$AlSi_2O_5OH$) respectively and were prepared by using a multiple pellet sampler. Each 150 mg pellet contains 1mL drug. Sterilized distilled water mixed with pyrophyllite regarded as control. Homeopathic drug pellets and untreated pellets were dried aseptically under the laminar air flow hood.

**Nematode inoculum and extraction:** *M. javanica* population were obtained from the infected roots of tomato (*Solanum lycopersicum* L.) and identified by the perennial pattern (Taylor & Netscher, 1974). Eggs were obtained by using 2% sodium-hypochlorite solution (McClure *et al*., 1973). Suspension of eggs was poured on a submerged cotton-wool filter and incubated for 72 hours in which juveniles emerged out. These juveniles were collected and used as inoculums. Population of *M. javanica* was multiplied and maintained by culturing on tomato roots present in a greenhouse (Department of Botany, University of Karachi).

**Experimental setup:** Field experiment was conducted in Department of Botany, Karachi University and designed properly as 2.5 x 2.5 micro-plots. Six homeopathic drug pellets (900mg) of *Kent*-20 and *Santonine*-43 amended in the soil respectively. Pellets containing sterilized distilled water regarded as control. Water it for at least 2 days for the decomposition of pellets, so it spread evenly throughout the soil. Five untreated seeds of mung bean (*Vigna radiata* (L.) R. Wilczek. cv. NM-2006), okra (*Abelmoschus esculentus* (L.) Moench cv. Arka anamika), sunflower (*Helianthus annuus* L. cv. Hysun-38) and mash bean (*Vigna mungo* (L.) Hepper cv. NM-97) were sown in each plot separately (4 ft furrows) which after sowing covered with soil. Treated and non-treated seeds were sown in sandy loam soil of a field containing sand (74%), silt (16%) and clay (10%) having pH 7.6 and organic matter, 1.24%. Plots were watered regularly until seedling was one week old. After that, freshly hatched *M. javanica* (second stage juveniles approximately 2000) were inoculated near roots of each seedling for each treatment. Plot without treatment was regarded as control. Each treatment was replicated 4X in a complete randomized block design (CRBD). Daily water and observed the plants till it reached the fruiting stage and uprooted the plants to record the growth and infection parameters on test crops.

**Collection of data:** The growth parameters includes; shoot and root length (cm), shoot and root weight (g), number of seeds and nodules as well as seed and flower weight (g) were recorded. For infection data produced by *M. javanica* nematodes were numbered of galls formed on the whole root system, egg masses per root system, eggs and egg mass were also examined. The number of galls and number of egg masses developed for the entire root system by *M. javanica* were counted under a low magnification. For observation of eggs/egg mass, ten egg masses from each treatment of all replicates were selected randomly from the root. Each egg mass was crushed in a drop of sodium hypochlorite solution (1%) to dissolve the gelatinous matrix and observe under a light microscope (De Leij, 1992).

**Statistical analysis:** Data of measurements were analysed by two way analysis (ANOVA) as per experimental design followed by the least significant difference (LSD) test at P=0.05 and Duncan's multiple range (DMR) test to compare treatment means, using statistical software (Sokal & Rohlf, 1995).

## Results

A field experiment was conducted to study nematicidal activity of homeopathic drug pellets against *M. javanica*. Six homeopathic pellets of 75% concentration of both drugs respectively when applied to soil increased the length and weight of plant but also showed maximum number of nodules on the roots of mung bean plant. However, maximum seed weight was attained by the amendment of 75% *Kent*-20 pellets followed by *Santonine*-43. By the addition of *Kent*-20 pellets (50% concentration) in soil, reduced number of galls produced by *M. javanica*/root system, number of egg masses and eggs/egg mass on mung bean roots significantly ($p<0.01$; 0.001), while 50% concentration of *Santonine*-43 showed minimum effect on inhibiting the galls formation in contrast to control (Fig. 1). In case of mash bean plants, significant ($p<0.001$; 0.05) enhancement in shoot length, shoot weight, root length, number of nodules and seed weight, while root weight showed non-significant attained by the incorporation of 75% concentration of both homeopathic drug pellets followed by 50% concentration. When comparing, 75% *Kent*-20 pellets gave healthy plant growth followed by *Santonine*-43 pellets. Seed weight was increased significantly ($p<0.001$) due to the inoculation of homeopathic pellets in all concentrations. It was found that number of galls/root system, number of egg masses and eggs/egg mass on mash bean roots were reduced by amendment of 75% concentration of *Kent*-20 pellets, followed by *Santonine*-43 pellets which also decreased infection of *M. javanica* by suppressing the galls produced on the roots of mash bean plant (Fig. 2). All concentrations of homeopathic pellets improved the growth and yield of okra. On the other hand, the effect was more distinct when soil was amended with 75% concentration of *Kent*-20 and *Santonine*-43 followed by 50% concentration ($p<0.001$). Progressive increased in okra plant growth was assessed by all treatments used. However, highest weight was recorded when soil was amended with 75% concentration of *Kent*-20

pellets. Number of galls/root system and egg masses was suppressed to utmost level when soil was amended with six pellets of *Kent*-20 and *Santonine*-43 (75% concentration) as compared to 50% concentration. The most adverse effect on growth parameters produced by nematode infection was recorded by untreated homeopathic pellets (Fig. 3). In case of sunflower plants, greater shoot length ($p<0.01$) and shoot weight ($p<0.001$) recorded by *Santonine*-43 pellets in contrast to *Kent*-20 which showed highest root length and weight at 75% concentration ($p<0.001$). All concentrations of homeopathic drug pellets contributed towards the maximum weight of flower. Soil amendment with 75% concentration of both homeopathic drug pellets markedly decreased gall formation; egg masses/root system and egg masses followed by 50% concentration as compared to control (Fig. 4).

Overall results showed that used of homeopathic pellets at different concentrations were found more efficient in increment of growth parameters and reduction of root knot infection. However, 75% concentration of *Kent*-20 pellets was more progressive in decreasing the galls formation by root knot nematodes, whereas *Santonine*-43 was best in improvement of growth of crop plants. Furthermore, 50% concentration of both homeopathic drug pellets was effective and has much potential for improvement of crop plants which were equally effective, while 75% *Kent*-20 pellets was found best in contrast to other treatments for controlling root knot infection and improved the growth of crop plants.

**Discussion**

In the present research work, all the concentrations of homeopathic drug pellets when inoculated in the soil separately showed improvement in the plant growth of tested crops. Homeopathic drug pellets when amended in the soil decompose evenly, which releases nematicidal compound into soil which reduced the infection of root knot nematodes which helps tested crop plants to show better growth. Of the different drugs and concentrations used, *Kent*-20 with the concentration of 75% gave excellent results in growth enhancement and control of nematode infection. Hajihassani *et al.,* (2013) reported that the larvae of the nematode when infest to the roots of plant reduces yield. Root knot nematodes especially *Meloidogyne* spp., produced severe damage to crops by producing galls on roots showing symptoms of nematode feeding lead to the reduction in supply of water and nutrients from the soil and the plants showed stunted growth and wilting. Different steps for the management of nematodes were taken in which application of organic amendment recorded as a beneficial method due to the improvement in plant nutrients, permeability, biological activity and aeration in the soil (D'Addabbo, 1995). Hanif & Dawar (2018) reported that leguminous (mung bean and mash bean) and non leguminous (sun flower and okra) seeds treated with *Santonine*-43 and *Kent*-20 at 75% concentration and soil drenched with nematicides (furadan and tenekil) at 0.1% respectively, showed complete suppression of nematode population as well as no gall formation produced by *M. javanica* was observed. But on the other hand, these nematicides application are not only toxic to the root-knot nematodes, but often lead to disturbance in the environment and even create the depletion of the stratospheric zone (Choi *et al*., 2007). Environmentalists and consumers are strongly against with increased use of agrochemicals like nematicides in crop production (Kapkavalci *et al*., 2009). Resistant condition in the host plant is important against plant parasites (Tyler, 1933). Homeopathic drug pellets were amended in the soil to reduce the infection caused by *M. javanica*. Amendment of organic material when decomposed in the soil produced greater resistance potential in the host plants against the attack of plant nematodes (Oka *et al.*, 2007) and improved the nutritional requirements of the host (Karssen & Moens, 2006). Pellets prepared from *Santonine*-43 and *Kent*-20 using pyrophyllite produced significant declines in gall formation per root system which also leads to decrement in egg masses and eggs/egg mass on leguminous and nonleguminous plants. Particularly *Kent*-20 at 75% v/w concentration showed marked reduction in nematode galls as compared to *Santonine*-43. Incorporation of pellets prepared only with pyrophyllite (hydrous aluminum silicate) failed in controlling of *M. javanica* infection, which is similar to the study of Lewis & Papavizas (1991) in which alginate pellets unable to control the pathogenic activity. Several reports on the application of organic amendment used to provide remarkable results in the increment of plant growth and impressively suppress plant pathogens including fungi, bacteria and nematodes (Ali *et al*., 2001; Ikram & Dawar, 2013; Hanif & Dawar, 2015). *A. marina* and *R. mucronata* when applied to soil as capsules and pellets at different ratios, not only enhances plant growth parameters, but also reduces the severity of root diseases caused by plant parasitic nematode when assessed on okra and mung bean crops (Tariq & Dawar, 2015). Homeopathic medicines such as *Cina* (prepared from the flowering meristems of *Artemisia nilagirica* (Clarke) pamp and *Aakashmoni* (prepared from the funicles of *Acacia auriculiformis* A. Cunn) were highly effective in ameliorating mulberry diseases of root-knot (*Meloidogyne incognita*), leaf spot (*Cercospora moricola*), powdery mildew (*Phyllactinia corylea*) and tukra disease (*Maconellicoccus hirsutus*) as well as improved the plant growth (Datta & Datta, 2011). Swarowsky *et al*., (2014) and Sukul *et al*., (2013) reported the effectiveness of potentized *Cina* reduced the number of root galls, eggs and second-stage juveniles ($J_2$) of *Meloidogyne incognita* as well as improved the growth of vegetables.

Present research demonstrated that all the homeopathic drugs pellet showed satisfactory results, but *Kent*-20 used at 75% v/w concentration found to be more active in enhancement of growth and reduction in gall formation caused by *M. javanica* showed alternative ways of control, therefore it is suggested that soil amendment with homeopathic pellets produced healthy seedlings as it suppressed the nematode infection which protect the root functions from being attacked by plant parasitic nematode which feeds on it, as compared to control (non-amended with homeopathic pellets) which produced galls on roots affects the nutrient uptake from the soil and water supply causing wilt which results in the death of plants. These cost-effective homeopathic pellets found biodegradable, non-phytotoxic and non-pollutant beneficial to agriculture farming.

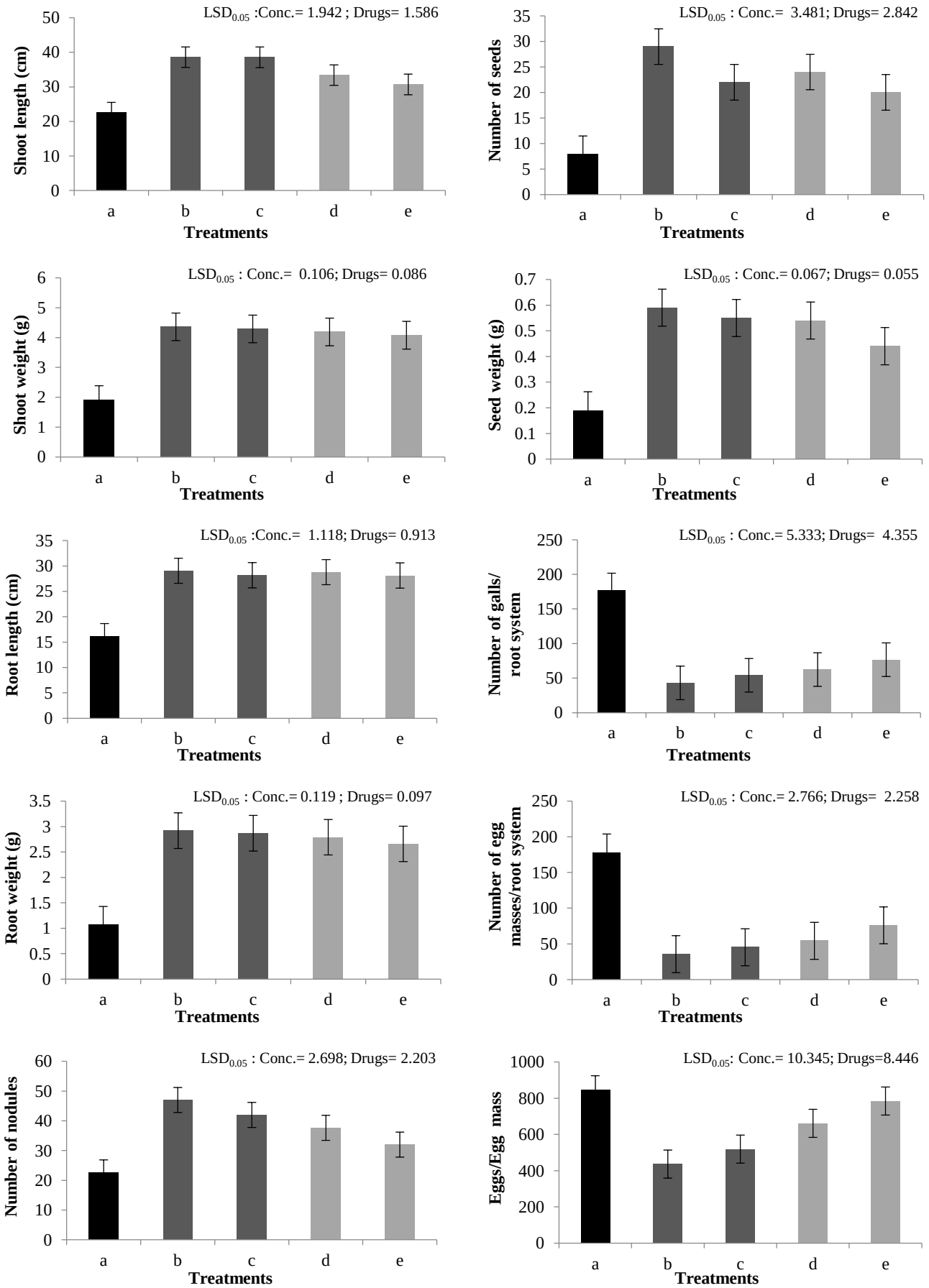


Fig. 1. Soil amendment with homeopathic drug pellets on growth promotion of mung bean against root knot nematode under field condition where, Conc. = Concentrations, a= Control, b= *Kent*-20 (75%), c= *Kent*-20 (50%), d= *Santonine*-43 (75%), e= *Santonine*-43 (50%) concentrations

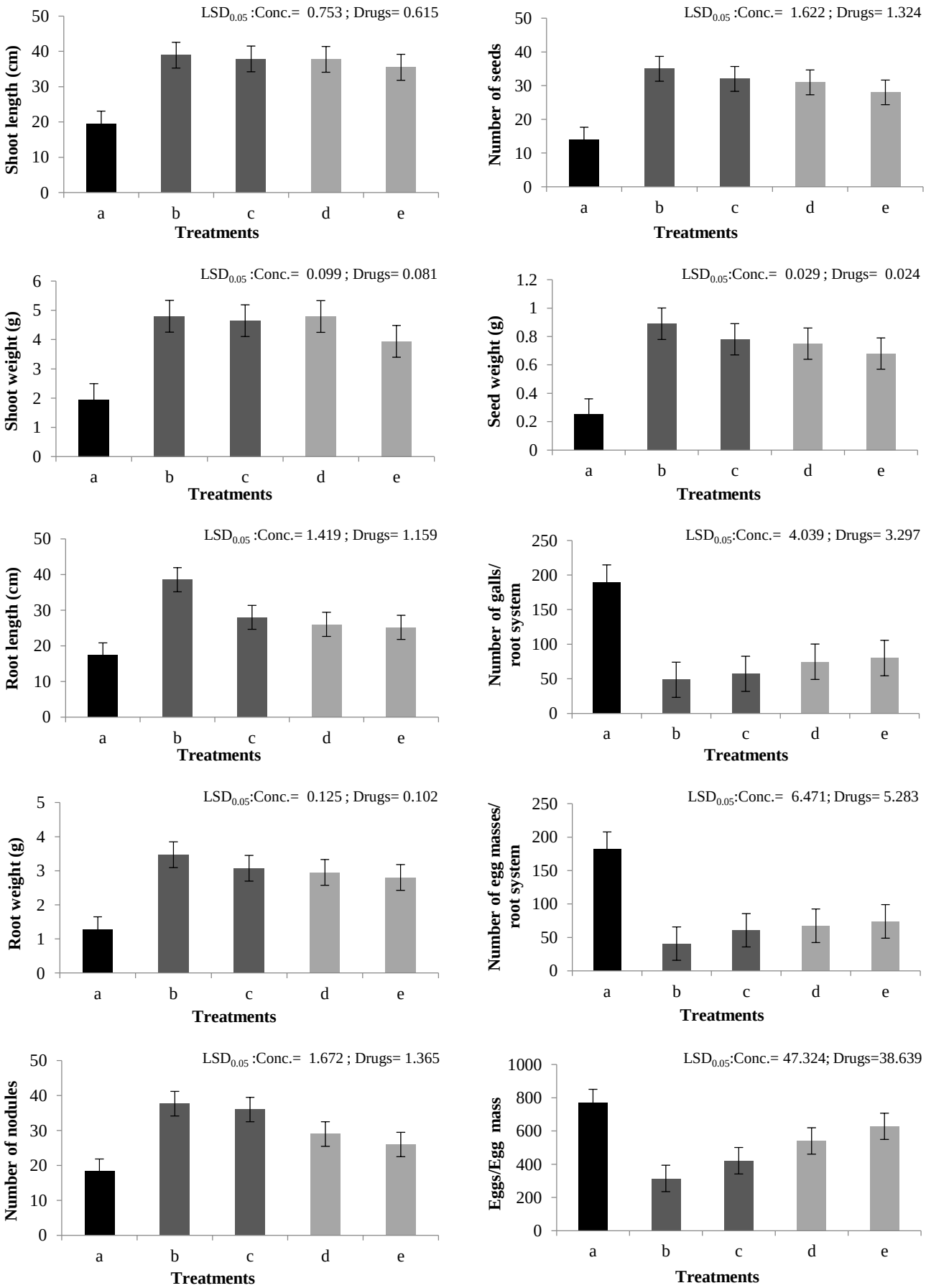


Fig. 2. Soil amendment with homeopathic drug pellets on growth promotion of mash bean against root knot nematode under field condition where, Conc. = concentrations, a= Control, b= *Kent*-20 (75%), c= *Kent*-20 (50%), d= *Santonine*-43 (75%), e= *Santonine*-43 (50%) v/v concentrations.

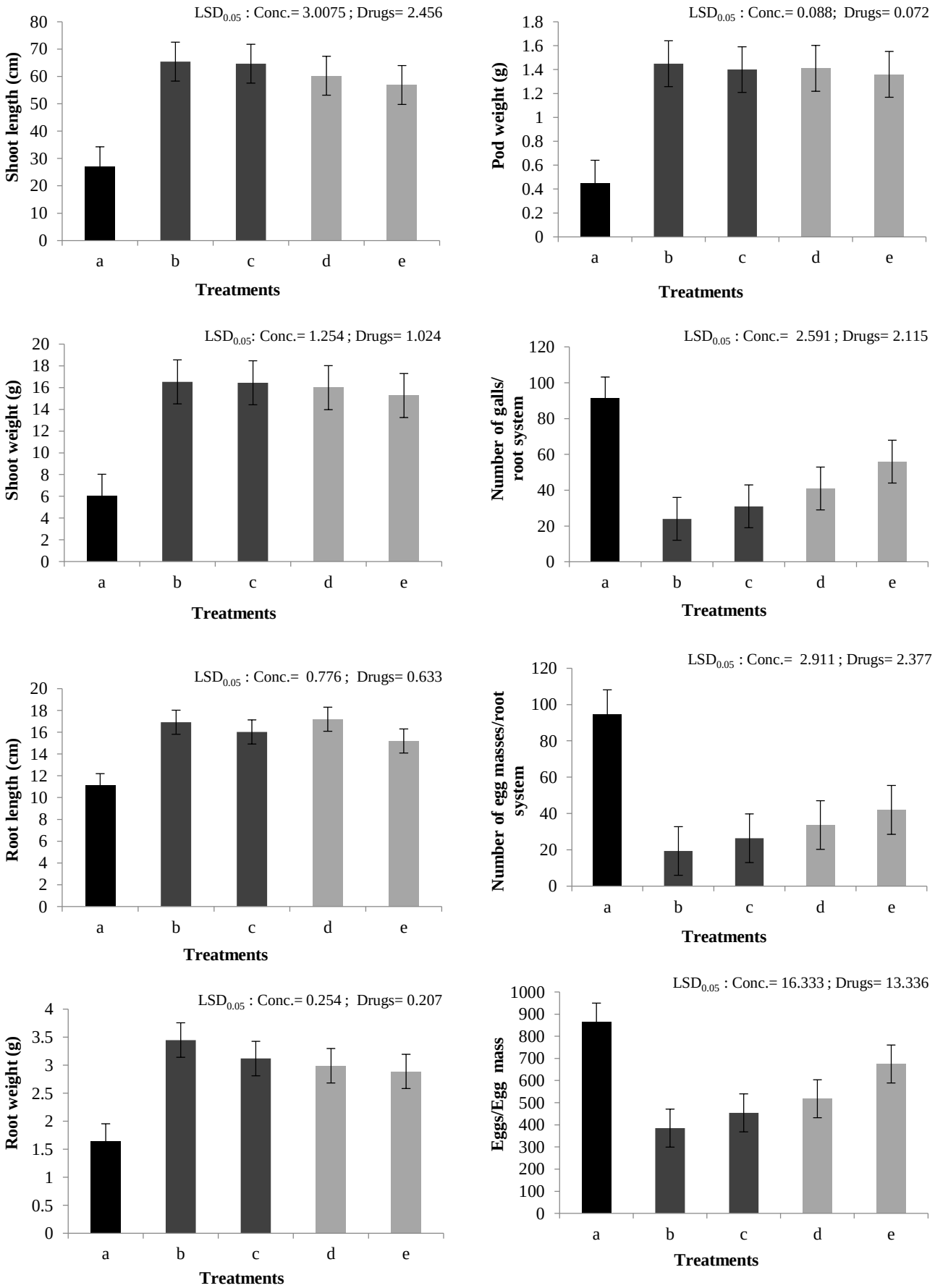


Fig. 3. Soil amendment with homeopathic drug pellets on growth promotion of okra against root knot nematode under field condition where, Conc. = concentrations, a= Control, b= *Kent*-20 (75%), c= *Kent*-20 (50%), d= *Santonine*-43 (75%), e= *Santonine*-43 (50%) concentrations.

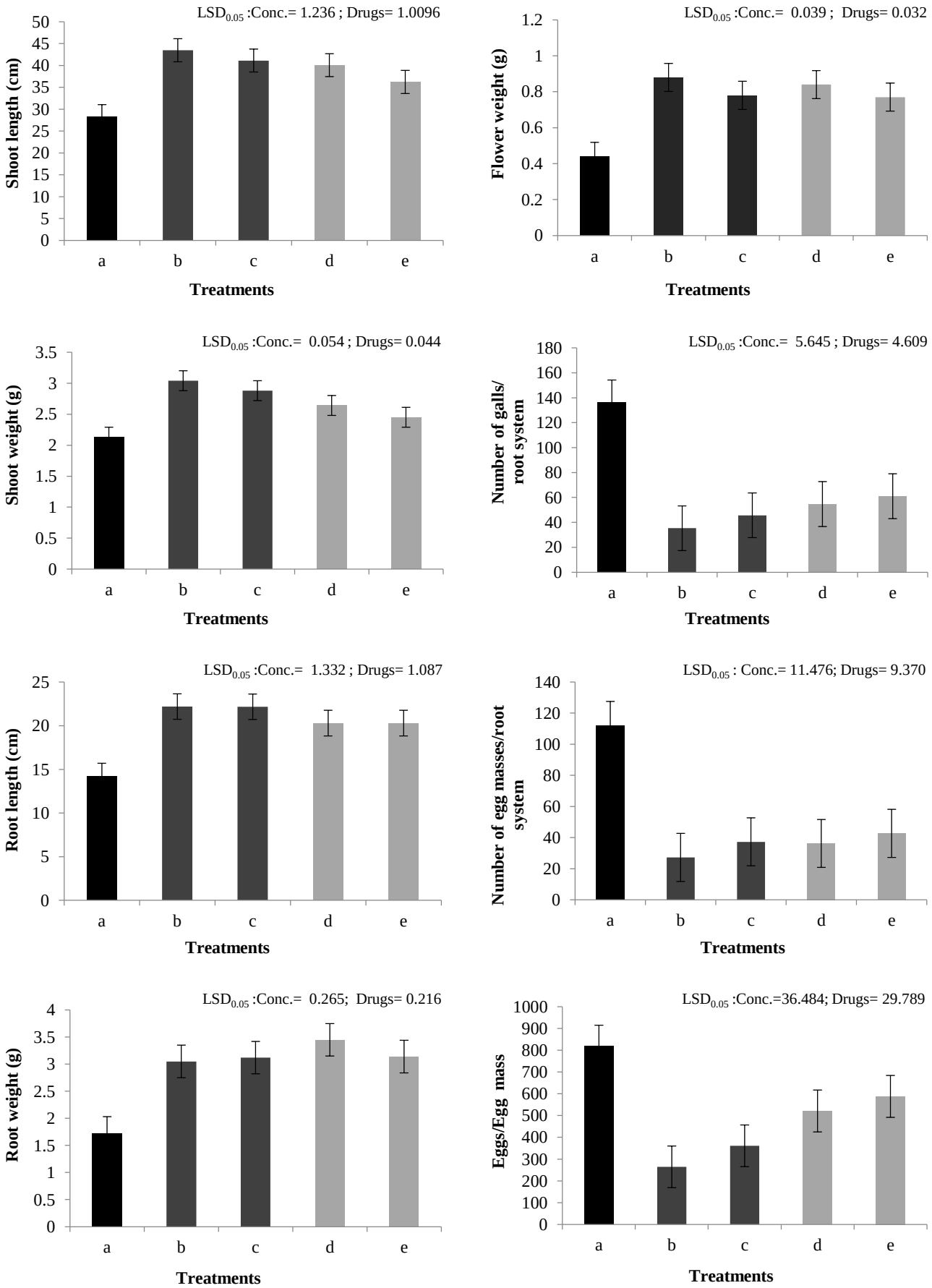


Fig. 4. Soil amendment with homeopathic drug pellets on growth promotion of sunflower against root knot nematode under field condition where, Conc. = concentrations, a= Control, b= *Kent*-20 (75%), c= *Kent*-20 (50%), d= *Santonine*-43 (75%), e= *Santonine*-43 (50%) concentrations.

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