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# Effect of sugar beet plant residues on population density of root knot nematode, *Meloidogyne incognita* infecting cowpea and biochemical changes in treated plants

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**Abstract**

Different sugar beet residues, fresh and dry leaves and mashed storage roots of sugar beet @20 and 10g were tested for controlling root knot nematode, *Meloidogyne incognita* on cowpea in screen house. On the basis of the average percentages nematode reduction, mashed storage roots at the highest rate (20g) achieved 85.1% increase followed by dry leaves at the same rate caused the reduction 81.6%. Average nematode reduction (79.5%) was caused by using fresh leaves of sugar beet at the highest rate followed by that occurred by the lowest one compared to untreated control. Plant growth, number of nodules (produced by nitrogen fixing bacteria, *Rhizobium*) and yield followed the same trend as the highest rate of sugar beet residues was used; there was the highest percentages increase of plant growth, yield and number of nodules. It is clearly noticed that soluble carbohydrates, total carbohydrates, phenols and soluble proteins in seeds increased at the different treatments compared to those of the untreated check and the effect, in general, was higher by using the highest rate compared to the lowest one. On the other hand, the contents of chlorophyll a, chlorophyll b and carotenoids in leaf increased at untreated check compared to those at different treatments.

**Keywords**: Sugar beet residues, dry and green leaves, tubers, root-knot nematode, cowpea.

Many researchers utilized certain plant part residues as organic soil amendments for controlling root knot nematodes (Youssef & Amin, 1997; El-Nagdi *et al*., 2011; Youssef & Lashein, 2013; Biondo *et al*., 2014). El-Sherbiny & Awd-Allah (2014) reported that powders of leaves of sugar and table beet reduced some nematode parameters in roots, final population and reproduction factor of *M. incognita* on tomato and subsequently plant growth parameters were improved in relation to untreated check. Youssef & El-Nagdi (2010) showed that dry leaves of sugar beet recorded the greatest percentage reduction (80.4%) for galls due to *M. incognita* on banana roots. This investigation was designed to determine effectiveness of mashed fresh and dry leaves and storage roots of sugar beet at different rates on the infectivity of root-knot nematode *M. incognita* on cowpea plants and to note plant biochemical changes.

## Materials and Methods

In this experiment, 5 kg solarized sandy loam soil (1:1 w/w) in each pot was prepared in a screen house on 30/6/2015. The following treatments of sugar beet (*Beta vulgaris* L.) residues were mixed with soil before cowpea sowing for decomposition:
1-10 g mashed fresh leaves of sugar beet
2-20 g mashed fresh leaves of sugar beet
3-10g mashed dry leaves of sugar beet
4-20g mashed dry leaves of sugar beet

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5- 10g mashed storage roots of sugar beet
6-20 g mashed storage roots of sugar beet
7-Sugar beet plants as untreated inoculated control.

Seeds of cowpea (*Vigna unguiculata* (L.) Walp) cv. Baladi seeds were sown at the same pots on 7/7/2015, seven days after adding different treatments to soil. Three seeds were sown in each pot at a depth of 2 cm; plants were thinned to one plant per pot, seven days after emergence to ensure uniform plant vigor. Then, 750 freshly hatched juveniles (J2) of *M. incognita*/pots were inoculated in each pot in holes around root system. Five replicates were used for each treatment. Harvesting was done for plants on 9/10/ 2015, 100 days after inoculation. The soil was sieved and decanted (Barker, 1985) for nematode extraction. Roots were gently cleaned by tap water to clean it from adhering soil particles and divided into two parts; the first part was put in petri dishes and stained by acid fuchsin lactophenol (Franklin & Goodey, 1949). Gall and egg-mass numbers on roots of cowpea were estimated using binocular microscope (Mai *et al*., 1996). The second part of roots was incubated in tap water according to Young (1954) for estimating number of the hatched juveniles from egg-masses. Plant growth criteria and number of nodules were recorded. Average percentages nematode reduction for each treatment was calculated to compare among treatments. Plant growth and yield vigor indices were calculated as averages plant growth or yield vigor criteria. The percentage plant growth or yield vigor index increase was calculated for each treatment compared to untreated control. Carbon/nitrogen (C/N) ratio for sugar beet residues was illustrated in Table 1. Total phenolic compounds were extracted from dry seeds and determined color metrically using Folin Ciocalteu phenol reagent according to the procedures described by Snell & Snell (1957). The method of Bradford (1976) was used for the assessment of soluble protein. Total soluble carbohydrates and total carbohydrates were determined in the dry seeds using the colorimetric method according to Dubois *et al*., (1956). Photosynthetic pigments (chlorophyll A (CA), chlorophyll B (CB) and carotenoids in the fresh leaves were determined according to Moran (1982).

Statistically, LSD test was used to analyze data. Comparisons among treatments were done by Duncan`s Multiple Range Test (DMRT) by using COSTAT programme version 4.

## Results

### Effect of sugar beet residues on nematode population

Table 2 indicates the significant ($P \leq 0.05$) influence of sugar beet residue treatments on nematode parameters of root-knot nematode on cowpea. It was obvious that the used sugar beet residues reduced nematode parameters as indicated by number of nematode juveniles in soil and roots, and galls and egg-mass numbers on roots of cowpea. On the basis of average percentages nematode reduction, there was a positive correlation between average percentages nematode reduction and the tested rates of each material, as the highest rate of treatment occurred; there is a higher average percentage nematode reduction. On this basis, mashed storage roots at 20g achieved 85.1% average reduction followed by dry leaves at the same rate when caused the reduction, 81.6%. Average nematode reduction (79.5%) was caused by using fresh leaves of sugar beet at 20 g followed by those occurred by the lowest one and untreated control.

**Table 1. C/N ratio of sugar beet residues.**

| Plant material | C/N |
|---|---|
| Sugar beet leaves | 13.8/1 |
| Sugar beet stems or tuber | 40.0/1 |

### Effect of sugar beet residues on plant growth parameters and number of nodules

Table 3 indicate the significant ($p \leq 0.05$) efficacy of the different residues on plant growth criteria and number of nodules as indicated by plant height, weights of shoots and roots and nodule numbers. On the basis of the average percentages plant growth vigor index increase, it was found that dry leaves at 20g caused the highest average plant growth increase index (74.4%) followed by mashed storage roots at 20 g, as it achieved 53.4% and dry leaves at 10g achieved 43.0 % average increases followed by those occurred by the lowest rates compared to untreated control. The least average increase (9%) occurred by fresh leaves at 10g. At the same trend, the number of nodules significantly increased according to the tested rates.

**Effect of sugar beet residues on plant yield criteria**

Table 4 indicates the significant ($p \leq 0.05$) efficacy of the different treatments on plant yield criteria as indicated by number and weight of pods/plant, weight of seeds/pod and weight of 100 seeds. On the basis of the percentage plant yield vigor index increase, it was found that dry leaves of sugar beet at 20g caused the highest increase (75.41%) followed by mashed roots at 20g (70.20%) and dry leaves at 10 g (64.84%) followed by those occurred by the lowest rates compared to untreated control.

**Effect of sugar beet residues on the biochemical compounds in cowpea plants**

Soluble carbohydrates, Total carbohydrates, Phenolics and Soluble proteins as affected by different treatments of sugar beet residues are presented in Table 5. It is clearly noticed that all contents increased at the different treatments compared to those of the untreated check and the effect, in general, was higher by using the highest rate compared to the lowest one.

**Effect of sugar beet residues on some photosynthetic pigments**

Chorophyll A and Chlorophyll B and Carotenoid contents as affected by different treatments of sugar beet residues were illustrated in Table 6. It was clearly noticed increasing these contents at untreated check compared to those at different treatments. However, the effect of the tested residues was higher at the highest rate than those occurred by the lowest rates.

## Discussion

In this experiment, different sugar beet plant parts either green and dry leaves or beet roots proved to be significantly reductive against root knot nematode, *M. incognita* on cowpea. This may be due to that leaves of beet root had a valued content of phenolic compounds (Biondo *et al*., 2014) toxic to nematodes (Ohri & Pannu, 2010). Mroczek *et al*., (2012) indicated that some red sugar beet (*Beta vulgaris*) roots contain 11 saponins and varying sugar numbers. This expression as reported in the literature by Mroczek *et al*., (2012) that may influence nematodes. Korayem & Salem (2000) showed that saponins at different extract concentrations of plants producing saponins could control root knot nematode and enhance plant growth. As reported by Ibrahim & Srour (2013), saponins reduced numbers of root-knot nematode which due to decrease in cholesterol level in nematode eggs.

In general, the exact mode of action of organic materials may be refer to either secondary toxic products resulted during decomposition of the tested materials containing some active ingredients which improve plant growth (Mahmood & Saxena, 1992). Besides, the C: N ratio of the amendment is an important factor in reducing nematodes especially when it is less than 20:1 (Agbenin, 2004), which may be due to decomposed toxic by-products.

However, in this experiment, less nematode reduction (79.5%, 81.6%) were achieved by using fresh and dry leaf residues at 20g, although they had the lowest C/N ratio (13.8:1) which may be due to faster decomposition of residues at the early stage of plant growth. The highest average nematode reduction (85.9%) caused by storage root residues which had the highest C/N ratio (40.0:1) may be due to slow decomposition of storage roots with prolonged residues effect.

**Table 2. Population density of *M. incognita* infecting cowpea as affected by mashed fresh and dry leaves and storage roots of sugar beet.**

| Treatments | Rate (g/pot) | $J_2$in soil | %Red. | Hatched $J_2$in roots | %Red. | No. galls | %Red. | No. egg-masses | %Red. | Average percentage nematode reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| **Green leaves** | 10 | 440c | 71.8 | 133c | 75.8 | 26b | 52.7 | 15b | 65.9 | 66.6 |
| | 20 | 200d | 87.2 | 53d | 90.4 | 19c | 65.5 | 11bc | 75.0 | 79.5 |
| **Dry leaves** | 10 | 567b | 63.7 | 152bc | 72.4 | 17c | 69.1 | 9cd | 79.5 | 71.2 |
| | 20 | 427c | 72.6 | 67d | 87.8 | 10d | 81.8 | 7cd | 84.1 | 81.6 |
| **Storage roots** | 10 | 433c | 72.2 | 180b | 67.3 | 14cd | 74.5 | 8cd | 81.8 | 74.0 |
| | 20 | 220d | 85.9 | 67d | 87.8 | 9d | 83.6 | 6d | 86.4 | 85.9 |
| **Untreated inoculated** | - | 1560a | - | 550a | - | 55a | - | 44a | - | - |

-Figures represent means of five replicates. Similar letter(s) of each column are not significantly different from each other on the basis of (DMRT) at 0.05 level

**Table 3. Plant growth of cowpea infected with the root-knot nematode, *Meloidogyne incognita* as affected by mashed green and dry leaves and storage roots of sugar beet.**

| Treatments | Rate (g/pot) | Plant height (cm) | Fresh weight of shoots (g) | Dry weight of shoots (g) | Fresh weight of roots (g) | No. of nodules | Plant growth vigor index | %Plant growth vigor index increase |
|---|---|---|---|---|---|---|---|---|
| **Green leaves** | 10 | 39.8d | 38.5f | 10.4 | 7.2a | 30c | 24.0 | 9.0 |
| | 20 | 50.5c | 44.2e | 12.6cd | 7.8a | 43a | 28.7 | 30.1 |
| **Dry leaves** | 10 | 52.3bc | 52.2c | 15.4b | 6.8a | 33bc | 31.7 | 43.0 |
| | 20 | 54.8ab | 71.1a | 20.7a | 7.8a | 40ab | 38.6 | 74.7 |
| **Mashed storage roots** | 10 | 52.5bc | 49.0d | 11.2de | 7.5a | 28c | 30.0 | 32.6 |
| | 20 | 55.8a | 58.4b | 13.9bc | 7.5a | 35abc | 33.9 | 53.4 |
| **Untreated inoculated** | - | 38.5d | 34.7g | 7.3f | 7.9a | 32bc | 22.1 | - |

-Values are averages of five replicates. Dissimilar letter(s) of each column are significantly different according to Duncan's Multiple Range test at 0.05 level. Plant growth vigor index= average plant growth criteria.

**Table 4. Plant yields of cowpea infected by *M. incognita* as affected by mashed fresh and dry leaves and storage roots of sugar beet.**

| Treatments | Dose (g/pot) | Pods/plant No. | Weight (g) | Seeds/pod No. | Weight (g) | Weight of 100 seeds (g) | Plant yield vigor index | %Plant yield vigor index increase |
|---|---|---|---|---|---|---|---|---|
| Green | 10 | 8c | 4.6de | 6cd | 0.66d | 10.56e | 5.96 | 21.14 |
| leaves | 20 | 9bc | 5.2d | 6cd | 0.69cd | 10.80d | 6.34 | 28.86 |
| Dry leaves | 10 | 12a | 9.6ab | 7bc | 0.77c | 11.17c | 8.11 | 64.84 |
| | 20 | 12a | 10.0a | 8ab | 0.91b | 12.22a | 8.63 | 75.41 |
| Storage | 10 | 9bc | 8.5c | 8ab | 0.88b | 10.95d | 7.47 | 51.83 |
| roots | 20 | 11ab | 9.1bc | 9a | 1.00a | 11.72b | 8.36 | 70.20 |
| Untreated inoculated | - | 5d | 3.9e | 5d | 0.57e | 10.13f | 4.92 | - |

-Figures represent means of five replicates. Similar letter(s) of each column are not significantly different from each other on the basis of (DMRT) at 0.05 level. Plant yield vigor index= average plant yield criteria.

**Table 5. Effect of mashed fresh and dry leaves and storage roots of sugar beet on some biochemical compositions of cowpea plants infected by *M. incognita*.**

| Treatments | Rate (g/5kg soil) | Soluble carbohydrates % | Total carbohydrates % | Phenolic content mg/g | Soluble proteins mg/g |
|---|---|---|---|---|---|
| Green leaves | 10 | 2.42a | 62.70bc | 33.69ab | 22.02cd |
| | 20 | 2.60a | 62.95bc | 34.09a | 23.47bc |
| Dry leaves | 10 | 2.21a | 63.24bc | 27.09d | 23.20bc |
| | 20 | 1.61b | 65.02a | 29.64c | 24.36b |
| Sorage roots | 10 | 1.53b | 64.05ab | 34.07a | 22.17cd |
| | 20 | 1.53b | 63.59abc | 31.96b | 27.11a |
| Untreated inoculated | - | 1.28b | 62.13c | 26.08d | 20.66c |

-Figures represent means of five replicates. Similar letter(s) of each column are not significantly different from each other on the basis of (DMRT) at 0.05 level.

**Table 6. Effect of mashed fresh and dry leaves and storage roots of sugar beet.on CA and CB and carotenoids of cowpea plants infected by *M. incognita*.**

| Treatments | Rate (g/5kg soil) | CA | CB | CA+ CB | Carotenoids (mg/l) | Total |
|---|---|---|---|---|---|---|
| Green leaves | 10 | 1.77bc | 0.57bc | 2.34bc | 0.28ab | 2.62bc |
| | 20 | 2.40a | 0.86a | 3.24a | 0.28ab | 3.52a |
| Dry leaves | 10 | 1.34c | 0.84a | 2.18c | 0.23c | 2.42c |
| | 20 | 1.95b | 0.64b | 2.59b | 0.25bc | 2.84b |
| Roots | 10 | 1.75bc | 0.58bc | 2.33bc | 0.28ab | 2.81b |
| | 20 | 1.90b | 0.62b | 2.52b | 0.29ab | 2.81b |
| Untreated inoculated | - | 2.23a | 0.80a | 3.04a | 0.33a | 3.37a |

-Figures represent means of five replicates. Similar letter(s) of each column are not significantly different from each other on the basis of (DMRT) at 0.05 level. CA= Chorophyll A, CB= Chorophyll B

Other factors were reported to induce greater nematicidal activity during decomposition of plant residues that plant tissues must be thoroughly chopped, mashed or powdered before their incorporation into soil and sufficient soil moisture (Morra & Kirkegaard, 2002) and optimum temperature (Ploeg & Stapleton, 2001; Lopez-Perez *et al*., 2005) should be provided at the same time of their incorporation.

As for biochemical changes in cowpea seeds, the studied biochemical compounds increased at the different treatments according to the tested rates which conform with the results obtained by El-Nagdi *et al*., 2014 and Youssef *et al*., 2015. On the other hand, photosynthetic pigments of cowpea leaves did not increase at the different treatments which may be explained that photosynthetic pigments were exploited for producing higher cowpea yields at the end of its growing season.

Either fresh and dry leaves or storage roots of sugar beet proved to be significantly reductive against root-knot nematode, *M. incognita* on cowpea which refer to that either leaves of beet root had a valued content of phenolic compounds or stems contain 11 saponins consisting of some effective materials against nematodes. The achieved yields using organic husbandries were encouragingly high and would have been considered so even in conventional husbandry.

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