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Effect of sugarcane molasses and ash on the organic management of root-knot nematode *Meloidogyne javanica* in tomato

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Abstract
The research was conducted to find out the effect of sugarcane molasses and ash on the organic management of root knot nematodes under screen house conditions. Sugarcane molasses were used @ 10 ml/kg, 20 ml/kg and 30 ml/kg of soil and ash @ 10 g/kg, 20 g/kg and 30 g/kg of soil twenty-one days before transplantation. Both the organic amendments were found effective against root-knot nematode. However, sugarcane molasses was more effective than ash and showed better results in terms of shoot and root lengths, fresh and dry shoot weight of tomato plant. Both the amendments (i.e. the sugarcane molasses and ash) significantly reduced galls plant^{-1} (41.40 and 48.4), galling index (3.00), egg masses/plant root system (23.40 and 28.20), fresh root weight (12.24 and 14.34 g) and dry root weight (4.89 and 5.73 g). Molasses and ash also significantly enhanced fresh shoot weight (60.20 and 57.40 g), dry shoot weight (10.36 and 9.12 g), shoot length (83.80 and 75.20 cm) and root length (26.20 and 24.00 cm) of tomato respectively. Sugarcane molasses and ash have been found with no toxic effect on plants.

Keywords: Organic management, root knot nematode, sugarcane molasses, ash

Introduction
In Pakistan, tomato (*Lycopersicon esculentum* Mill.) is one of the most important solanaceous crops. Tomato crop is very versatile and especially in developed countries, consumption of this crop is ever-increasing quite rapidly. A branded tomato extract, tomato is used for the treatment of high blood pressure. It is highly prized for its monitory gain and nutritional value especially for its richness in vitamins and minerals [1].

Tomato originated in South America and is widely cultivated in 140 countries of the world with an annual production of 150 million tonnes/hectares [2]. The world major tomato producing countries are China and USA followed by India and Turkey [3] In Pakistan, the total area under cultivation of tomato is 52 thousand hectares with the production of 0.5296 million tons, while Khyber Pakhtunkhwa shares an area of over 12 thousand hectares with a production of 0.2132 million tons [4].

This crop is attacked by a number of organisms such as fungi, bacteria, viruses and nematodes etc. The nematode is a very destructive pest of tomato which has caused much destruction to this important crop. Root knot nematode (*Meloidogyne* spp) is endo-parasitic nematode, economically important and has a large number of different species. Four important species of root knot nematodes (*M. javanica, M. arenaria, M. incognita, M. hapla*) are distributed worldwide and considered as major crop-damaging pests [5]. The average losses caused due to root-knot nematode infestation are about 20.6% in tomato but yield losses caused by root knot nematodes have been recorded from 20% to 33% [6]. Root-knot nematodes are obligate parasites and are capable of feeding inside the roots of over 2000 plant species, causing severe crop losses worldwide [7]. Heavy root galling are the characteristic symptoms of *Meloidogyne* spp. and got its common name root knot nematodes [6].

Cultural practices like crop rotation are used for controlling such diseases but in the case of soil borne pathogens these practices fail due to polyphagous nature of these parasites and can survive in soil for years. These parasites break the resistance of the resistant cultivars due to the introduction of virulent strains [8]. The existing management strategies can be improved by the development of organic agriculture [9]. Root-knot nematodes can be managed effectively by different means like chemical management, biological control agents and the addition of organic matter will decrease nematode population in the soil [10]. Synthetic chemical develops resistance in the pathogens and hazardous effect on the environment, today's demand for

environment-friendly pesticides with low toxicity and short-term persistence [11]. Therefore, the alternate management strategies are needed for controlling these plant parasites.

Biocidal effect of various products such as essences, essential oils, and aqueous extracts, have been reported on fungi, weeds and bacteria [13] as well as insects that reside in the soil. Natural plant products have the ability to provide a potential, alternative to synthetic chemicals that are used for the control of soil-borne pathogens and parasites, such as plant-parasitic nematodes [14].

The use of agro-industrial wastes in bioprocesses at one hand provides an alternative for a sustainable equilibrium of natural organic material, and on another side also helps in solving pollution problem that their disposal may cause. The initial population of these parasites significantly affect growth and yield of tomato [15]. The plant growth is inversely affected by the population of root-knot nematode [16].

The addition of organic residues has a strong impact on the physical and biological properties of soils and promotes an environment favorable to nematode-antagonistic microorganisms [17]. Incorporation of plant parts or extracts into the soil alone or with bio-control agents have also been suggested as an alternative, safe and effective control method for the management of plant parasitic nematodes [18]. The present study was conducted to manage root-knot nematode with sugarcane molasses and ash.

Materials and Methods

Collection of sugarcane molasses and ash

The sugarcane molasses and ash of crushed sugarcane were obtained from local Ghur factory of Utmanzai, Charsadda District, Khyber Pakhtunkhwa province of Pakistan.

Sugarcane molasses and ash were applied at three levels 10, 20, 30 ml/kg and 10, 20, 30 g/kg of soil in each pot respectively including with the controls. Each treatment has five replicates.

Collection of root knot nematodes

The infected plant roots were obtained from the fields of Dargai and Jaban areas of Malakand Division, where there was an immense infestation of root-knot nematode on tomato crop. The unthrifty and the plants showing stunting were uprooted and examined. The roots having galls were collected in polyethylene bags and were brought to the Plant Pathology laboratory, The University of Agriculture Peshawar.

Perineal pattern morphology of root knot nematodes

Perineal pattern morphology was followed for the identification of root knot nematodes [19]. Visible knots on the roots of tomato were crushed using a sterile needle in a petri dish containing distilled water. 10-15 females were randomly selected and each female was transferred to a glass slide and its posterior end was detached under a stereomicroscope. The perennial pattern was obtained by cutting the posterior cuticle and was transferred to clean glass slide in a drop of glycerin. The cover slip on the slide was sealed with paraffin. The slide was observed under a compound microscope using 100x with emulsion oil to identify *Meloidogyne* spp. [20]. The process was repeated for all the females. The nematode identified was *Meloidogyne javanica* shown in (Fig. 1).

Fig 1: Perineal pattern of *M. javanica* (100x)

Fig 2: Tomato nursery

Fig 3: Galled roots of tomato plant

Fig 4: Sugarcane molasses

Root-knot nematodes culturing
After identification, single mature egg mass of known *Meloidogyne javanica* was inoculated into the rhizosphere of three weeks old susceptible seedlings of Rio Grande variety of tomato in pots that were filled with pasteurized potting mixture in order to get mass pure culture. New tomato seedlings of the same cultivar were inoculated with 10-15 egg masses that were obtained from the pure culture for sub-culturing, in order to get sufficient inoculum for screening tomato germplasms experiment.

Raising of tomato nursery
For raising the tomato nursery, the seeds of Rio Grande variety of tomato (*Lycopersicon esculentum*) were obtained from Agriculture Research Institute Tarnab, Peshawar and were sown in pots containing pasteurized soil.

Application of soil organic amendments and transplantation
The pots were filled with pasteurized potting mixture of 1:1:1 (clay, silt, and FYM). The organic amendments were then mixed on the top six inches layer of soil in pots except for control. The pots were then labeled as according to the soil amendments applied. The pots were then covered with plastic sheaths and placed on concrete benches. Seedlings of uniform thrift and uniform heights were transplanted to each pot, twenty-one days after the application of soil organic amendments.

Root-knot nematodes inoculation
Known numbers of eggs were inoculated into the rhizosphere of the plants in each pot. The inoculation procedure of [21] was followed and so, 2000 eggs in 10 ml water in glass beaker were poured evenly around the stem of each tomato seedling per pot except control pots after ten days of transplantation in the screen house. All the agronomic practices were done normally for the growth and development of the plants.

Data collection
About seven weeks after inoculation, the plants were carefully uprooted, and roots were washed gently and the data were recorded on various parameters:

Number of galls/plant root system
The number of galls on the entire root system of the uprooted tomato plants was counted and means of all the replicates of the individual plant was calculated.

Galling index
Galling index was assessed on a scale of 0-5 as described by [22] as follow:
0 = No gall on roots
1 = 1-2 galls
2 = 3-10 galls
3 = 11-30 galls
4 = 31-100 galls
5 = More than 100 galls.

Number of egg masses/plant root system
The uprooted tomato roots were then stained for twenty minutes in an aqueous solution of Phloxine B (15mg per liter) as reported by [23] The number of egg masses per plant root was then counted under a stereoscope.

Number of eggs/egg mass
The eggs were released from the gelatinous matrix by applying 1.0% NaOCl [24]. Ten egg masses were randomly selected and a number of eggs per egg mass was assessed and the data was recorded for analysis.

Statistical analysis
Completely randomized design (CRD) with two factors was used in the screening experiment with five replications and controls. Averages of all the recorded data were estimated and were subjected to statistical analysis using the technique of analysis of variance and for mean comparison least significant difference (LSD) test was used [25]

Layout for screen house experiment
Factor 1:- M= Molasses, A= Ash
C_0= Negative control (no inoculation and no amendments), C_1= Positive control (inoculated but no amendments), C_2 and C_3= Amendments applied but no inoculation (C_2M_1, C_2M_2, C_2M_3 and C_3A_1, C_3A_2, C_3A_3).
Factor 2:- Doses
For Molasses: M_1= 10 ml/kg, M_2= 20 ml/kg, M_3= 30 ml/kg
For Ash: A_1= 10 g/kg, A_2= 20 g/kg, A_3= 30 g/kg

M_2	C_2M_1	A_1	C_3A_3	M_2	A_1	A_3	A_1	C_2M_1	A_3	C_3A_2	C_0	A_2	C_2M_2
C_3A_1	A_2	C_3A_1	A_2	C_2M_3	C_1	C_1	C_1	C_3A_3	A_3	C_3A_1	M_2	C_0	A_3
C_0	M_3	M_3	C_1	C_3A_2	M_3	C_2M_1	M_3	C_2M_1	M_1	A_2	M_2	C_2M_2	C_2M_2
C_3A_2	C_0	C_2M_2	M_1	A_1	C_3A_2	C_3A_3	M_2	C_3A_3	C_3A_3	M_1	C_2M_3	A_1	A_3
C_2M_3	C_1	C_3A_2	C_2M_1	C_2M_2	C_3A_1	C_2M_3	A_2	M_1	M_1	C_2M_3	C_0	M_3	C_3A_1

Results
The study was aimed to have an easy and cost effective organic management of tomato root knot nematode (*Meloidogyne javanica)* according to existing farming system and to introduce environment and friendly techniques to manage root-knot nematode problem in tomato growing areas of Khyber Pakhtunkhwa. The results of the current research are given as follows:

Identification of root knot nematodes
Ten randomly selected females of root-knot nematode were processed to study the perineal pattern of root knot nematode. From perineal pattern morphology it was confirmed, that the species identified was of *Meloidogyne javanica* shown in Fig. 1.

Effect of sugarcane molasses and ash on galls/plant of tomato.
Significant effects (P<0.05) were observed with the application of organic amendments and their interaction on the number of galls/plant of tomato (Table 1). All the application doses significantly reduced galls/plant. The maximum reduction in galls/plant was recorded by the plants amended with sugarcane molasses (i.e 41.40), followed by the plants amended with ash (i.e 48.40) under root knot inoculated conditions. Sugarcane molasses significantly (P<0.05) reduced galls plant^{-1} with the highest dose (i.e 30 ml/kg) being the most effective (i.e 26.40) followed by the highest dose of ash (i.e 30.03) of the same dose. The maximum galls/plant was observed with no application of amendments under inoculated conditions.

Effect of sugarcane molasses and ash on the galling index of tomato.

Results showed a reduction in galling index with different doses of sugarcane molasses and ash applied and their interactions (Table 2). The maximum reduction in galling index was recorded by sugarcane molasses (i.e 3.40), followed by ash (i.e 3.60). The greatest reduction in galling index was recorded by the highest dose (i.e 30g or ml/kg)

Table 1: Effect of sugarcane molasses and ash on galls plant^{-1} of tomato.

Treatments	Application doses			Means
	10 g or ml kg^{-1}	20 g or ml kg^{-1}	30 g or ml kg^{-1}	
Molasses (Treatment+ Inoculation)	67.00 c	30.80 e	26.40 f	41.40 c
Ash (Treatment+ Inoculation)	69.00 b	48.00 d	28.20 f	48.40 b
C1 (Only inoculated)	101.40 a	101.40 a	101.40 a	101.40 a
C2 (MOLASSES) (No Inoculation)	0.00 g	0.00 g	0.00 g	0.00 d
C3 (ASH) (No Inoculation)	0.00 g	0.00 g	0.00 g	0.00 d
C0 (No inoculation, No treatments)	0.00 g	0.00 g	0.00 g	0.00 d
Means	39.56 a	30.03 b	26.00 c	

LSD $_{(organic\ amendments)}$ = 1.10
LSD $_{(application\ doses)}$ = 0.78
LSD $_{(interaction)}$ = 1.92

Table 2: Effect of sugarcane molasses and ash on the galling index of tomato.

Treatments	Application doses			Means
	10 g or ml kg^{-1}	20 g or ml kg^{-1}	30 g or ml kg^{-1}	
Molasses (Treatment+ Inoculation)	4.00 b	3.20 c	3.00 c	3.40 c
Ash (Treatment+ Inoculation)	4.00 b	4.00 b	3.00 c	3.60 b
C1 (Only inoculated)	4.60 a	4.60 a	4.60 a	4.60 a
C2 (MOLASSES) (No Inoculation)	0.00 d	0.00 d	0.00 d	0.00 c
C3 (ASH) (No Inoculation)	0.00 d	0.00 d	0.00 d	0.00 c
C0 (No inoculation, No treatments)	0.00 d	0.00 d	0.00 d	0.00 c
Means	2.10 a	1.96 b	1.76 c	

LSD $_{(organic\ amendments)}$ = 0.17
LSD $_{(application\ doses)}$ = 0.12
LSD $_{(interaction)}$ = 0.31

of sugarcane molasses and ash (i.e 3.00). Different doses significantly caused a reduction in the galling index of sugarcane molasses at 20 ml/kg (i.e 3.20), followed by dose (20g/kg) of ash (3.20). The maximum galling index (i.e 4.60) was recorded with no application of organic amendments under inoculated conditions.

Effect of sugarcane molasses and ash on egg masses/plant root system of tomato.

Results from Table 3 revealed that egg masses/plant root system were significantly (P<0.05) reduced by organic amendments, their interaction and their increasing application doses. Significant reduction in egg masses/plant root system were shown by the plants amended with sugarcane molasses (i.e 23.40) at a dose (30ml/kg) of soil, followed by the plants amended with ash (i.e 28.20) at dose 30g/kg of soil. The increasing application doses had a significant effect on egg masses/plant root system. The highest dose of 30g or ml/kg of soil was the most effective (i.e 25.53) under the screen house condition. The maximum egg masses/plant (i.e 101.60) was observed by the plants that were not amended under inoculated conditions.

Effect of sugarcane molasses and ash on eggs/egg mass of tomato.

Eggs/egg mass were significantly reduced with the organic amendments applied as shown in Table 4. The increase in the application doses significantly reduced eggs/egg mass. The maximum reduction was recorded for the highest dose of sugarcane molasses (i.e 278.00), followed by ash (i.e 286.00) of the same dose. The increase in eggs/egg mass (i.e 296.20) was observed in plants with no amendments application under inoculated conditions.

Table 3: Effect of sugarcane molasses and ash on egg masses/plant root system of tomato.

Treatments	Application doses			Means
	10 g or ml kg^{-1}	20 g or ml kg^{-1}	30 g or ml kg^{-1}	
Molasses (Treatment+ Inoculation)	33.40 c	26.20 d	23.40 e	27.66 c
Ash (Treatment+ Inoculation)	36.20 b	31.20 c	28.20 d	31.86 b
C1 (Only inoculated)	101.60 a	101.60 a	101.60 a	101.60 a
C2 (MOLASSES) (No inoculation)	0.00 f	0.00 f	0.00 f	0.00 d
C3 (ASH) (No inoculation)	0.00 f	0.00 f	0.00 f	0.00 d
C0 (No inoculation, No treatments)	0.00 f	0.00 f	0.00 f	0.00 d
Means	28.53 a	26.50 b	25.53 c	

LSD $_{(organic\ amendments)}$ = 1.29
LSD $_{(application\ doses)}$ = 0.91
LSD $_{(interaction)}$ = 2.24

Table 4: Effect of sugarcane molasses and ash on eggs/egg mass of tomato.

Treatments	Application doses			Means
	10 g or ml kg^{-1}	20 g or ml kg^{-1}	30 g or ml kg^{-1}	
Molasses (Treatment+ Inoculation)	290.00 bc	284.00 e	278.00 f	284.00 c
Ash (Treatment+ Inoculation)	292.00 b	288.00 cd	286.00 de	288.67 b
C1 (Only inoculated)	296.20 a	296.20 a	296.20 a	296.20 a
C2 (MOLASSES) (No Inoculation)	0.00 g	0.00 g	0.00 g	0.00 d
C3 (ASH) (No Inoculation)	0.00 g	0.00 g	0.00 g	0.00 d
C0 (No inoculation, No treatments)	0.00 g	0.00 g	0.00 g	0.00 d
Means	146.37 a	144.70 b	143.37 c	

LSD $_{(organic\ amendments)}$ = 1.49
LSD $_{(application\ doses)}$ = 1.05
LSD $_{(interaction)}$ = 2.59

Discussion

The world is moving towards the organic agriculture these days because of the ill effects of chemicals. This research focused on finding out different cheap and cost effective organic management strategies for controlling root-knot nematode thereby avoiding costly and hazardous chemicals. Chemicals are not much effective in controlling nematodes because nematodes are protected in hard egg shells. Chemicals are also costly and most of the farmers cannot afford. Secondly, huge quantities of organic wastes (agricultural wastes) are wasted every year. These organic wastes can be re-used and converted into wealth.

This research aimed to find the effect of molasses and ash on the management of root-knot nematodes and also to recycle these wastes. This study revealed that molasses and ash both had a significant effect on the management of root-knot nematodes and plant growth. This research has explored cost effective, environment and farmer friendly methods for the management of nematodes.

Sugarcane molasses also stimulated the shoot and root length, fresh and dry shoot weight of tomato, and suppressed galls/plant, galling index, eggs/eggmass, and egg masses/plant root system. The dose level of 30g or 30 ml/kg of the soil of both amendments were found more effective. Sugarcane molasses proved more effective than ash. [26] Also reported a reduction in root galling in tomato by the application of molasses to the soil [27]. Reported that addition of molasses to the soil time to time increased the sugarcane yield, especially in low potassium areas. The application of molasses to the field plots in relatively higher rates, controlled root knot more effectively as compared to nematicide fenamiphos [28].

The addition of molasses, which is rich in carbon, can reduce the number of nematodes even more than amending the soil with urea alone [29]. Organic amendments, such as molasses are environment-friendly and do not pose any threat to the environment as other chemical pesticides do because chemical pesticides readily decomposed into the soil to CO_2 and other harmless organic products.

Ash is applied to the soil for plant growth improvement, as it increases the aeration and also improves the soil structure. [30] reported that organic amendments such as grass ash and rice husk ash proved to be beneficial means of controlling root-knot nematode in soil. They recommended that the farmers should be convinced to use these organic amendments because the chemicals used have adverse environmental effects and health hazards.

My results are in line with the results of as the application of ash improved the plant height and suppressed gall/plant. The effect of ash on plants may be indirect. Ash improves the texture, structure, aeration and water holding a capacity of soil required for plants. Some micronutrients may also be there which promotes the growth of plants, however, further studies are required to confirm the possibilities.

Different kinds of amendments used as a nutrients source for crop production have been found effective in controlling root diseases of plants. The application of ash also effectively reduced galls/plant, galling index, eggs/egg mass, egg masses/plant root system and also fresh and dry weight of galled roots as compared to controls. Plant height and fresh and dry weight of shoot of plants were increased. Further, it also inhibited nematodes population [31].

These studies also revealed that un-inoculated and amendments added plants showed the highest performance and stimulated plant growth. The sugarcane molasses performed better than ash in this research. Hence, the results showed that there must be some kind of nutrients or growth promoting factors in the amendments used and needs further studies to identify and confirm.

This research has contributed a lot to the science and explored possibilities to recycle the organic wastes, wasted every year. Molasses and ash not only manage root knot-nematodes but also increase the organic contents and fertility of soil which is need of the country. Also, the environmental pollution shall be greatly reduced. The methods are also farmer friendly and farmers can themselves use their wastes in their crops.

Conclusion

Sugarcane molasses and ash both were found effective to manage root knot nematodes; however, application of sugarcane molasses gave better results.

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Author Contributions

Mehran Khan under supervision of Prof. Dr. Saifullah conceived, designed, performed the experiment and this research was part of his MPhil thesis. Aqleem Abbas analyzed the data, edited and wrote the paper. The other two authors Ijaz Ahmad and Rifat Ali Khan contributed materials and analysis tools.

Conflict of Interest

The authors have no conflict of interest to declare.

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