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Protecting cucumber from *Meloidogyne incognita* using graft onto resistant cucurbit rootstocks and antagonistic marigold as an alternative to nematicide

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Abstract

The effect of cucumber grafting onto suitable and selected two rootstocks, wax melon, *Benincasa hispida* (grafted onto *Bh*) and *C. maxima* x *C. moshata* Ercola hybrid 6001 (grafted onto Ercola 6001), marigold (*Tagetes* spp.) as root antagonistic plant and Rugby as a nematicide (two formulation, Rugby 10G and Rugby 20L) for control of *Meloidogyne incognita* cucumber root-knot were evaluated in nematode naturally infested soil under greenhouse conditions in two successive spring seasons (2011 and 2012). Cucumber, *Cucumis sativus* var. Sinai was planted as a scion. Results indicate that cucumber plants grafted onto Ercola hybrid 6001, *C. maxima* x *C. moshata* (Ercola 6001) and *Bh* had highly significant less root galling, number of females and egg-masses than non-grafted and infested one. The reduction in number of galls ranged between Rugby 10G (47%) grafted onto *Bh* (98.3%) and Rugby 10G (30.2%) grafted onto Ercola 6001 (84.5%), respectively in two seasons followed by grafted onto Ercola 6001 and marigold and finally Rugby 10G. These results indicated that maximum reduction in number of females and egg-masses forming was on cucumber grafting onto resistant plant rootstocks *Bh*; grafting onto resistant plant rootstocks Ercola 6001, followed by Rugby 20L, marigold and finally Rugby 10G, respectively as compared by nematode infested cucumber. Root-knot nematode (RKN) significantly decreased shoot plant height and leaf area, when they were grown in soil infested by RKN compared to infested plant (check). In addition, RKN infestation alone decreased the plant nematodes, in natural infested soil experiments in both seasons, data was non-significant. Plant and fruit characteristics; cucumber yields (number of days to first flower, fruit characteristics (fruits weight, length and diameter), total early fruits (first four gathering) and total fruit per plant were significantly improved in plant leaves compared with infested cucumber. Consequently, the shoot macro and micro-elements were increased compared with infested cucumber. The macro and micro-elements and chlorophyll contents were recorded but the variation between them were non-significant.

Root-knot nematodes, *Meloidogyne* species are the most wide spread on a wide plant hosts range including agronomic and vegetable crops, ornamental, fruit trees and weeds, especially in tropical and subtropical countries (Amin, 1994). In Egypt, root-knot nematodes are one of the most limiting factors in crop production (Oteifa & Tarjan, 1965; Amin, 1993). In vegetable crops production in greenhouses, most of the damage from continuous cropping is caused by soil-borne diseases and nematodes (Oda, 1999).

Current management of nematodes has been done by using plant resistance, crop rotation, culture practices or nematicides (Chitwood, 2002). Nematicides control is expensive and hazard to ground water, environment and animal and human health. Because of these reasons, we are looking for alternative safety application and methods such as antagonistic plant and grafting onto selected resistance rootstocks (Amin *et al*., 2013; 2012). Linford *et al*., (1938) studied the nematicidal effect of pineapple leaves as organic amendment against *Meloidogyne* spp. Some of the plant species antagonistic to *Meloidogyne* spp., are leaves and flowers of *Tagetes* spp., and many other plants like, neem, garlic, peppermint (Amin & Youssef, 1997).

Grafting of vegetables on resistant rootstocks is a mean of controlling root-knot nematodes and other soil borne diseases in areas with intensive land use (Lee, 1994; Greco, 1999; Ioannou, 2001;

Maršić & Osvald, 2004; Lopez-Perez *et al*., 2006; Amin *et al*., 2012).

In the present study, the effect of marigold on cucumber grafting onto suitable and selected two rootstocks and Rugby as a nematicide (two formulation, Rugby 10G or Rugby 20L) for control of *Meloidogyne incognita* cucumber root-knot were evaluated in naturally infested soil under greenhouse conditions.

Materials and Methods

Planting: The grafted plants and other treatments were grown in soil contaminated with the root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 (initial population 2 ± 1 juveniles/g soil). This experiment was carried out in the inspected greenhouse located at Kaha Research Station, Horticultural Research Institute, Agricultural Research Center (Qualiobia governorate, Egypt). Commercial cucumber scion, *Cucumis sativus* L., Sinai hybrid was grafted onto Wax melon, *Benincasa hispida* (*Bh*) and Ercola hybrid 6001 (*C. maxima* x *C. moshata*) Ercola 6001. The plug seedlings were grown in a greenhouse using routine cultivation techniques. Seedlings of rootstocks and scions were ready for grafting after seeds germination at the appearance of the second true leaf of rootstocks 10 days after sowing. Cucumber seedlings (used as scions) were grafted onto the rootstocks.

Grafting method: Seedling of rootstocks was picked between the two cotyledons after removing the top (leaves) by razor. Scion was prepared by cutting the seedling below the two cotyledons with a distance of 2-3cm with a basal end in the form of a wedge. Wedges were inserted in the picked part of the stocks on condition that the cotyledons of the rootstocks and scion were held with grafting clips (Oda, 1999). The scion and rootstock were held with a grafting clip and sprayed with water. Clips were removed 12-15 days after grafting. The seedlings were placed under plastic tunnel at optimum temperature and humidity. The compatibility was determined after 10 days at relatively low temperature (20-25 ^{o}C) in spring season from conducting after grafting stage (following the new growth on the scion). The plastic tunnel was gradually, opened for adaptation. Ten days after grafting, plants were transferred to greenhouse, and appropriate shading was provided under a mist propagation system for adaptation of grafted plants to the outside environment.

Two experiments were carried out in naturally infested soil by root-knot nematode, *M. incognita* in two successive spring seasons (2011-2012). Cumber, *C. sativa* var. Sinai was grafted onto wax melon, *Benincasa hispida* (*Bh*) and Ercola hybrid F1 6001, *C. maxima* x *C. moshata* (Ercola 6001) under greenhouse (40 m long x 9 m width x 3 m height). Four marigold plants were planted around every cucumber plant in treatment in one m^2 and Rugby as a in two formulation, Rugby 10G added at 5 g per one m^2 (one gram per plant), and Rugby 20L added with water irrigation at concentration of one ml/one litter tap water per one m^2 (200 ml/plant) and other plants were left as control in nematode infested soil. The soil texture of the experimental field was loamy clay. The plants under greenhouse were irrigated by drip irrigation and fertilized according to the recommendations of the Egyptians Ministry of Agriculture. The treatments were replicated three times (plot = one m^2) in a completely randomized block design. After nine weeks of transplant, roots of plants were carefully up rooted and nematodes in soil and roots were counted and recorded as root galls and egg-masses index according to Taylor & Sasser (1978). The number of galls, females and egg-masses and reproductive factor as compared to untreated plots was calculated for root-knot nematodes. Early yields and total yield/plant (kg) were calculated and recorded.

The greenhouse was layered in one longitudinal ridge (40 m) long and one meter width. Seedlings were planted on the two sides of each ridge at half m intervals. After 2 months old planting 20 plants were randomly chosen from each experimental plot. Plant height, leaf area and chlorophyll contents (spade) were recorded. Fruit

characteristics (fruit weight, fruit length and diameter), early yields/plant (The first four gathering) and total yield of cucumber per plant were recorded as well as N, P, K (macro elements) and Mg, Ca (micro elements) contents in plant shoot were analyzed using the modified method described by Plummer (1971). The number of galls, females and egg-masses and gall/egg-masses index and percentage of galls infected roots in plant were calculated for root-knot nematode.

Statistical analysis: Analysis of variance with SAS software (SAS Institute, Cary, NC) was carried out and mean treatments data difference (LSD) calculated at 0.05.

Results

The results of grafted cucumber, *Cucumis sativus* var. Sinai onto *Benincasa hispida*; grafted cucumber, *Cucumis sativus* var. Sinai onto Ercola hybrid 6001; marigold (4 plants around cucumber trunk); Rugby 10G and Rugby 20L (Table 1 in season 2011 and Table 2 in season 2012) showed significant differences ($p \leq 0.05$). The results indicated that all treatments and grafting methods had highly decreased the number of galls, females and egg-masses and consequently the galls/egg-masses index and percentage of root galling. The reduction in number of galls ranged between Rugby 10G (47%) grafted onto *Bh* (98.3%) and Rugby 10G (30.2%) grafted onto Ercola hybrid 6001 (84.5%) in two seasons. These results indicated that maximum reduction in number of females and egg-masses forming was on cucumber grafting onto resistant plant rootstocks *Bh* (one female and one egg-mass), grafting onto resistant plant rootstocks, Ercola 6001 (2.2 females and 1.5 egg-masses), Rugby 20L (9.1 female and 7.5 egg-masses), marigold, *Tagetes* spp., (10.9 females and 10 egg-masses) and Rugby 10G (13.2 and 10.8), number of females and egg-masses, respectively compared by nematode infested cucumber plant (65 females and 52.5 egg-masses) Figs. 1 and 2.

Results for gall and egg-masses index (Table 1 spring 2011) showed that all treatments and grafting had effect and lowest number between 1/1 (on cucumber grafted onto resistant plant rootstocks *Bh*) and 2/3 (marigold and Rugby 10G) compared by nematode infested cucumber plant (4/4). The same results in spring 2012 in agreement with spring 2011showed in Table 2.

Grafted cucumber onto *Bh*, grafted cucumber Ercola 6001, marigold (4 plant), Rugby 10G and Rugby 20L, applied to cucumber infested with *Meloidogyne incognita* influenced growth weights of early yield per plant (first 4 gathering) and total cucumber yield per plant in season 2011 and 2012. Significant differences between treated and untreated plants were recorded. The highest yield obtained per plant (Table 3 and Fig. 3) after grafted cucumber onto *Bh* (2.5 and 2.4 kg/plant) and Rugby 20L treatment (2.4 and 2.3 kg/plant) in spring seasons 2011 and 2012, respectively compared by infested cucumber (1.7 and 1.58 kg/ plant). The average of fruit length and diameter were increased significantly in all treatments in both seasons compared by infested cucumber in both season while fruit weight was not significant in most cases (Table 4). The increasing in plant height and leaf area of cucumber was significant compared with infected control in spring seasons 2011 and 2012 (Table 5).

Results showed not significantly effect on total chlorophyll content while number of days to first flower decreased between one to 9 days earlier flower when cucumber treated with Rugby, marigold or applied by grafting onto resistant plants in both seasons (Table 6). The macro and micro elements were recorded but the variation between them were not significant in both seasons (Table 7).

Table 1. Effect of marigold on two selected rootstocks grafted cucumber and Rugby on population of root-knot nematodes in spring season, 2011.

Treatments	Number of				GI/EI *	Galls infected root (%)
	Galls	Developmental stages	Females	Egg-masses		
Grafted onto *Bh*	1.0 f	1.0 e	1.0 d	1.0 d	1/1	1.0 c
Grafted onto Ercola 6001	5.0 e	1.0 e	2.2 d	1.5 d	1/1	5.1 e
Marigold	17.8 d	10.1 c	10.9 c	10.0 b	3/2	10.3 d
Rugby 10G	31.2 b	14.4 b	13.2 b	10.8 b	3/2	20.9 b
Rugby 20L	22.7 c	8.1 d	9.1 c	7.5 c	2/2	17.9 c
Cucumber in infested soil	58.9 a	14.9 a	65.0 a	52.5 a	4/4	32.7 a

Table 2. Effects of marigold on two selected rootstocks grafted cucumber and Rugby on population of root-knot nematodes in spring season, 2012.

Treatments	Number of				GI/EI	Galls infected root (%)
	Galls	Developmental stages	Females	Egg-masses		
Grafted onto *Bh*	10.8 c	1.0 c	1.0 d	1.0 c	1/1	1.0 c
Grafted onto Ercola 6001	13.1 c	1.0 c	3.0 d	2.0 c	2/1	6.3 c
Marigold	13.5 c	17.3 b	13.0 c	11.0 b	3/3	12.5 bc
Rugby 10G	48.5 b	26.8 a	21.0 b	17.0 b	3/3	23.4 ab
Rugby 20L	32.5 bc	24.6 a	14.3 bc	13.0 c	3/3	21.1 b
Cucumber in infested soil	69.5 a	16.0 b	73.0 a	60.0 a	4/4	34.6 a

Table 3. Effects of marigold on two selected rootstocks grafted cucumber and Rugby on early and total yields per plant in two spring seasons.

Treatments	Season 2011		Season 2012	
	Early yield g/plant	Total yield g/plant	Early yield/plant	Total yield/plant
Grafted onto *Bh*	813.3 b	2530 a	753.7 c	2.42 a
Grafted onto Ercola 6001	786.0 c	2100 c	713.3 d	2.35 ab
Marigold	623.3 d	1800 d	670.3 c	1.7 c
Rugby 10G	818.7 b	22 00 c	778.0 b	2.22 b
Rugby 20L	908.3 a	2400 b	891.0 a	2.30 ab
Cucumber in infested soil	577.0 e	1700 e	575.0 f	1.58 c

Table 4. Effects of marigold on two selected rootstocks grafted cucumber and Rugby on cucumber fruits criteria in two spring seasons.

Treatments	Season 2011			Season 2012		
	Average fruit weight (g)	Average fruit length (cm)	Average fruit diameter (cm)	Average fruit weight (g)	Average fruit length (cm)	Average fruit diameter (cm)
Grafted onto *Bh*	132.3 a	15.7 a	3.4 a	132.7 a	15.8 a	3.0 b
Grafted onto Ercola 6001	130.0 abc	15.3 a	3.4 a	129.7 b	15.0 b	3.1 bc
Marigold	129.0 bc	11.3 c	3.1 b	128.3 b	11.6 c	3.0 bc
Rugby 10G	131.6 ab	12.6 b	3.4 a	132.0 a	11.5 c	3.1 bc
Rugby 20L	130.6 ab	12.9 b	3.4 a	128.0 b	11.8 c	3.0 b
Cucumber in infested soil	127.7 c	10.1 c	3.1 b	123.7 c	10.0 d	2.9 c

Table 5. Effects of marigold on two selected rootstocks grafted cucumber and Rugby on plant height and leaf area in two spring seasons.

Treatments	Season 2011		Season 2012	
	Plant height (cm)	Leaf area (cm)	Plant height (cm)	Leaf area (cm)
Grafted onto *Bh*	273.7 b	374.0 b	277.3 b	354.3 b
Grafted onto Ercola 6001	282.7 a	384.0 a	295.0 a	374.7 a
Marigold	232.7 d	390 .0 cd	239.0 d	315.3 d
Rugby 10G	263.7 c	337.7 d	271.0 c	314.7 de
Rugby 20L	235.3 d	342.3 b	238.0 d	320.0 c
Cucumber in infested soil	228.7 e	328.7 e	239.0 d	311.7 e

Table 6. Effects of marigold on two selected rootstocks grafted cucumber and Rugby on chlorophyll level and number of day to first flower in two spring seasons.

Treatments	Season 2011		Season 2012	
	Chlorophyll	No. of days to first flower	Chlorophyll	No of days to first flower
Grafted onto *Bh*	42.9 a	31.7 b	48.7 a	31.6 b
Grafted onto Ercola 6001	43.8 a	27.6 c	42.2 ab	30.0 c
Marigold	36.5 b	36.0 a	38.4 b	37.3 a
Rugby 10G	38.5 b	35.3 a	42.5 ab	38.0 a
Rugby 20L	37.8 b	35.6 a	41.4 ab	38.0 a
Cucumber in infested soil	39.0 ab	36.0 a	39.6 b	37.6 a

Table 7. Effects of marigold on two selected rootstocks grafted cucumber on certain macro and micro elements in two spring seasons.

Treatments	Season 2011					Season 2012				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Grafted onto *Bh*	1670	352	1032	336	83	1678	348	1028	342	87
Grafted onto Ercola 6001	1589	351	1044	342	89	1583	358	1054	338	85
Marigold	1663	339	1049	321	72	1663	347	1053	339	73
Rugby 10G	1600	340	1052	318	71	1660	347	1050	240	75
Rugby 20L	1664	341	1048	320	74	1665	345	1051	338	71
Cucumber in infested soil	1666	340	1050	320	70	1600	349	1053	338	73

Discussion

These findings indicated that marigold and grafted cucumber onto selected rootstocks *Bh* and Ercola 6001 applied to cucumber infested with *Meloidogyne incognita* had anti-nematode activity equal or more than that recorded from Rugby 20L or Rugby 10G treatments. The results of these studies showed that marigold, *Tagetes* spp., plant had released nematicidal activity against nematodes that agreed with many naturally occurring compound that are known to posses nematicidal activity, polythienyes from *Tagetes* spp., (Kyo *et al*., 1990). In our study *Tagetes* spp., had nematicidal activity against *M. incognita* causing root-knot disease and agreed with results obtained by Katooli *et al*., (2011) and Amin & Youssef (1997).

Greenhouse results indicated that high reduction on number of galls, females and egg-masses that agreed with results obtained by Youssef & Amin (1997) and Rather & Siddiqui (2007), also agreed with experiment of Amin *et al*., (2013) in grafting onto resistant rootstocks to be effective against *M. incognita*. The nematicidal effect of tested *Tagetes* attributed to the high contents of certain oxygenated compounds, which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells (Knoblock *et al*., 1989; Katooli *et al*., 2011). Alam *et al*., (1978) reported that the volatile fatty acids and many others that released during decomposition of *Tagetes* have been reported to be toxic to nematodes.

The change in plant physiology due to the application of such chemical nematicides is possibly useful, whereas, it enables the treated plants to overcome nematode invasion and reproduction. Rugby 20L was most effective than formulation Rugby 10G with no significant difference. All treatments were effective in reducing nematode population.

It is concluded that cucumber grafting onto both scions were the most effective agent for decreasing nematode development, while marigold treatment suppressed nematode populations. The highest yields obtained per plant and recorded as a result of nematode reduction in both seasons. The average of fruit length and diameter was increased significantly in all treatments in both seasons compared by infested cucumber, while fruit weight was not significant in most cases. The difference in plant height and leaf area of cucumber was significant compared with infected control in both seasons. On the other hand, results showed no significant effect on total chlorophyll content while number of days to first flower decreased between one to 9 days earlier flower after treatments in both seasons. While, macro and micro elements were recorded but the variations were not significant in both seasons. From previous data which was recorded significant increase on plant growth and increasing yields in different treatments which are

conformable to their resistant to infestation with nematode and supported with Owens & Novothy (1960) that plant tissues infested by nematodes showed considerable increases in the levels of free amino acids and many chemicals. Increase in the activity of such chemicals is at least in part responsible for synthesis of auxins, hormones and many other compounds that are involved in the defense mechanisms of plant to nematodes. The use of grafting onto resistant cucurbit rootstocks has proved to be effective for control of *M. incognita* and other soil borne diseases. These studies will be helpful for alternative of grafting onto selected resistant plant and certain medicinal plants like *Tagetes* with expensive and harmful chemical nematicides against soil borne nematodes.

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