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Control of *Meloidogyne incognita* (Kofoid and White) Chitwood by Cadusafos (Rugby ®) on Tomato

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Abstract.- The cadusafos (Rugby ®) showed strong nematicidal property in lab experiment and greenhouse trail. Cadusafos 1%, 0.5% and 0.25% was tested in egg hatching, juveniles mortality and invasion and development of root knot nematodes *Melodigyne incognita* (Kofoid and White) Chitwood in tomato to (*Solanum lycopersicum* L.). Cadusafos 1% was found to be effective in egg inhibition of *Meloidogyne incognita.* Cadusafos (Rugby ®) 1% showed 100% while 0.5% and 0.25% showed 72% and 57.3% mortality of juveniles, respectively. Mortality of juveniles increased with increased exposure time and concentration. Invasion, development and reproduction of root knot nematode were evaluated on tomato crop. Data was recorded after 7, 17 and 35 days to observe the penetration and development stages including vermiform, swollen, sausage stage, immature females, adult females and egg masses of *M. incognita*. Maximum reduction of *M. incognita* was observed when soil was treated with Cadusafos (Rugby ®) 1%. Most effective concentration of cadusafos determined by above experiments was used to assess its effect as protective and curative. Root knot nematode population was reduced when cadusafos was applied as protective application.

Key words: Cadusafos, Rugby, root knot nematode, mortality, invasion and development.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important kitchen crop of Pakistan and is grown over 53150 hectares with average yield 9.93 tonnes/hectare. In Pakistan, the average yield of tomato is very low as compared to 76.13 tonnes/hectare in USA (FAO, 2008). Low yield might be due to the lack of quality seed, little choice of genotypes against different stresses, attack by number of insect pests and diseases. Root-knot nematode, *Meloidogyne* spp., reniform nematodes (*Rotylenchulus reniformis* Linford and Oliveira) and several other ectoparasitic nematodes have been found attacking tomato (Kamran *et al.,* 2010; Khan *et al*., 2011). Tomato is rated as the third most susceptible vegetable crop to root-knot nematode after aubergine and okra (Khan, 1993; Anwar *et al*., 2007; Rehman *et al*., 2009). Among the biological entities nematodes, particularly root-knot nematodes are wide spread and play a significant role in reducing the yield of vegetable crops (Khan *et al*., 2011). Root-knot nematodes infection of tomatoes limits fruit production by 30% (Anwar and McKenry, 2010). Among various control measures, use of chemicals has been considered as an effective strategy for control of plant parasitic nematodes when other methods like cultural practices, resistant varieties and biocontrol agents are unable to protect crops from these pests (Hague and Gowen, 1987; Barker and Koenning, 1998). Chemicals may play a major role to assure crop protection and production threatened by root-knot nematodes when other control measures are ineffective. Currently various nematicides and non-fumigants are available in the market against these pests (Rich *et al*., 2004). Granular non-fumigant nematicides are more easily applied and safer for farmers compared with fumigants (Lamberti *et al*., 2000). The most extensively used non-fumigant nematicides are aldicarb, cadusafos, carbofuran, ethoprop, fenamiphos, fosthiazate, oxamyl and terbufos, which are organophosphate or carbamate based nematicides.

Among the systemic nematicides, Temik in all application and Nemaphos as side drenching exhibited a fairly high nematicidal effect against *Meloidogyn*e spp. (Dl-Muro, 1970). Sivakumar *et al*. (1973) showed that while seed treatment with carbofuran may not give absolute protection against the root-knot nematode. However, Sivakumar *et al.* (1976) revealed the persistence of nematicidal activity in seed treatment of okra with carbofuran

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and aldicarb sulphone. Though, Sitaramaiah and Vishwakarma (1978) reported the relative efficacy of selected non-volatile nematicides *viz.*, fensulfothion, carbofuran, mocap, phorate and aldicarb at different doses in field for control of *Meloidogyne incognita* and *M. javanica* on okra and tomato.

Mahajan (1978) and Jain (1990) reported the efficacy of carbofuran for the control of *M. incognita* and *M. javanica*, respectively on okra. Evaluation of mephosfolan for nematode and insect control in okra, cowpea and tomato was revealed by Prasad *et al*. (1982). Reddy and Singh (1983) investigated that aldicarb applied at 1 kg a.i. /ha significantly reduced the population of *M. incognita* on okra, brinjal, french bean and cowpea. Adverse effect of phenamiphos on soil nematodes and resultant increased yield of cowpea, pea and okra was studied by Sethi and Meher (1989). In plant-parastic nematodes, acetylcholinesterase inhibition by crbamate and organophosphate nematicides was reported by Opperman and Chang (1990). Roy *et al*. (1990) screened nineteen O, O-diaryl O-2 chloroethyl phosphates for toxicity against *M. incognita*. Out of which three phosphates showed good nematicidal activity in vitro and further in treated soybean plants. Ten pesticidal chemical namely Thiride, Dithane M45, Bavistin, Aldrin 30 E.C., Monocrotophos, Thimet 10 G, Furadan 3 G, Phorate 10 G, Ziram and Satum were tested against *M. incognita* infesting soybean variety T-49. All the chemicals except Bavistin and Ziram were effective in reducing nematode larval population (Mishra and Gupta, 1991). Efficacy of carbofuran in control of root knot nematode was reported by Adegbite and Agbaje (2007) and Myjada *et al*. (2011) on yam and groundnut, respectively.

The root-knot nematodes (*Meloidogyne* spp.) infecting different vegetable crops are worldwide in its distribution. The present study was conducted to investigate the effect of cadusafos (Rugby ®) using different concentrations against *Meloidogyne incognita*.

MATERIALS AND METHODS

Effect of cadusafos on egg hatching

Three concentrations of cadusafos (Rugby ®) (1%, 0.5% and 0.25%) were evaluated. Egg masses of uniform color and sizes were picked with fine forceps from galled brinjal roots induced by *M. incognita*. Eggs were smashed in 1% sodium hypochlorite (NaOCl) solution to dissolve the gelatinous matrix and to release the eggs from egg masses (Hussey and Barker, 1973).

Fifty eggs were introduced in 6 ml of the cadusafos solution in a 5 cm diameter dish then each dish covered with lid. Each treatment was replicated fifteen times. These Petri dishes were randomized in a tray. Then they were kept in incubator at 28ºC.

Numbers of hatched juveniles were observed after 3, 6 and 9 days under microscope. The unhatched eggs were rinsed on 500 mesh sieve and were placed in clean 5cm diam. Petri dishes containing distilled water to observe if type of reaction of chemical on egg hatch. The emerging J2 were counted at 2, 4 and 6 days under stereoscope.

Effect of cadusafos on juvenile's mortality

The assessment was carried out in 5 cm diam. Petri plates containing 5 ml of different concentration of cadusafos (1%, 0.5% and 0.25%). Nematode suspension carrying 25 freshly hatched juveniles with the help of pipette was added. One Petri plate containing juveniles in water was kept as control. Each treatment was replicated fifteen times. These dishes were covered with lids and incubated at 28 ºC in an incubator.

Dead nematodes were counted under microscope after 12, 24, and 48 h. Ten to fifteen juveniles were transferred to sterilized distilled water to confirm their death. In case they did not regain mobility they were considered as dead (Khan *et al*., 2009). Percent J2 mortality was calculated by using the following formula:

$$\text{Percent juveniles mortality} = \frac{\text{Number of juveniles killed}}{\text{Total number of juveniles}} \times 100$$

Effect of cadusafos on invasion and development

Air dried sieved soil was treated with different concentration of cadusafos 1%, 0.5% and 0.25% and distributed into 100 ml small pots. Three days after adding soil 15 days old tomato seedlings were transplanted into these pots and they were completely randomized in growth room. The soil without chemical was kept as control. Each treatment was replicated ten times. One week after transplanting these plants were inoculated with 1000 freshly hatched juveniles. These pots were not watered immediately after inoculation. This experiment was conducted into three sets *viz.*, first and second set to be observed the invasion and development, the third for the reproduction of nematodes.

Data were recorded after 7, 17 and 35 days to observe the penetration and development stages including vermiform, swollen, sausage stage, immature females, adult females and egg masses of *M. incognita*. Roots were stained with Phloxin B for observation and counting of egg masses (Southey, 1986).

Protective and curative application of cadusafos

Most effective concentration of cadusafos determined by above experiment was used to assess its effect as protective and curative. One month old tomato seedlings were transplanted into these pots. The freshly hatched 1,000 J2 per plant were used as inoculum. Each treatment was replicated ten times. Cadusafos was applied before inoculation as protective and after inoculation as curative effect. One treatment without chemical was kept as control.

Data analysis

Data were subjected to ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA, 1988) and significant difference among the treatments was portioned by Duncan's multiple range test (LSD) at probability levels of P = 0.05 (Steel *et al*., 1997).

RESULTS

Effect of cadusafos on egg hatching

The results showed that distinct concentrations of cadusafos (1%, 0.5% and 0.25%) differed significantly from control in reducing egg hatching (Table I). It was observed that higher dose (1%) was effective in reducing hatching and it differed from lower doses (0.5% and 0.25%). It was observed that by increasing the dose of chemical the hatching was reduced. Hatching increased with the increase of time.

After one day, nematodes in cadusafos showed a highly significant difference over control. Similar results were observed after 3 and 6 days, respectively.

Table I.- Effect of cadusafos on egg hatching.

Treatments	Dosage	After 1 day	After 3 days	After 6 days
Cadusafos	1.00%	1.0 g*	1.7 g	1.7 g
	0.50%	4.3 g	8.0 f	9.3 f
	0.25%	16.0 e	23.7 d	28.3 c
Control	-	31.3 c	42.0 b	47.7 a

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

Effect of cadusafos on J2 mortality

The results indicated that maximum juvenile mortality was observed in the 1% followed by 0.5% and 0.25% after 12 h. Mortality of juveniles increased with increased exposure time and concentration (Table II). The 100% mortality was recorded at 1% cadusafos after 12 hwhereas after 48 h at 0.5% cadusafos. 0.25% cadusafos was least effective concentration showed 84.70% mortality after 48 h of incubation.

Table II.- Effect of cadusafos on J2 mortality.

Treatments	Dosage	12 h	24 h	48 h
Cadusafos	1.00%	100.0 a*	100.0 a	100.0 a
	0.50%	72.0 c	84.7 b	100.0 a
	0.25%	57.3d	72.3 c	84.7b
Control	-	0.0 e	0.0 e	0.0 e

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

Effect of cadusafos on invasion and development

At the first harvest after 7 days, minimum number of J2 invaded in 1% concentration of cadusafos followed by 0.5% and 0.25% (Table III). Two developmental stages i.e. vermiform and swollen were recorded at all concentrations (1%, 0.5% and 0.25%) but significantly lower as compared to control (with water only). At 1% cadusafos vermiform and swollen stages were recorded as 14.2 and 33.2, respectively as compared to control (51.8 and 342.4, respectively).

After 17 days swollen, sausage shape, immature and adult females were observed with highest number of immature females at all the concentrations but significantly lower than control (Table IV). Minimum invasion and development was observed in 1% concentration with swollen (3.2), sausage shape (11), immature (24.4) and adult females (3) significantly lower than control (51.4, 80.0, 1172.2 and 39.2, respectively). Vermiform was not observed after 17 days.

Table III.- Effect of cadusafos on the invasion and development of *M. incognita* on tomato after 7 days.

Treatments	Dosage	Developmental stages	
		Vermiform	Swollen
Cadusafos	1.00%	14.2 d*	33.2 d
	0.50%	21.0 c	90.2 c
	0.25%	34.8 b	179.2 b
Control	-	51.8 a	342.4 a

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

Table IV.- Effect of cadusafos on the invasion and development of *M. incognita* on tomato after 17 days.

Treatment	Dosage	Developmental stages			
		Swollen	Sausage	Immature females	Adult female
Cadusafos	1.00%	3.2 d*	11.0 d	24.4 d	3.0 d
	0.50%	21.6 c	26.0 c	53.2 c	13.8 c
	0.25%	34.8 b	52.0 b	123.0 b	29.0 b
Control	-	51.4 a	80.0 a	172.2a	39.2 a

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

After 35 days vermiform, swollen, sausage shape, immature females, adult females and egg masses were recorded significantly higher in control (Table V). 1% cadusafos show minimum number of vermiform (63), swollen (22), sausage shape (33), immature females (4.2), adult females (28.6) and egg masses (23.6) followed by 0.5% and 0.25% (Table V).

This showed clearly that cadusafos (Rugby ®) at all concentrations tested proved effective on invasion and development of root-knot nematode (*M. incognita)* as compared to control. 1% cadusafos (Rugby ®) show lowest invasion and development followed by the other concentrations (0.50% and 0.25%).

Effect of cadusafos as protective and curative application

Effect of cadusafos (Rugby ®) 1% on the development of root weight, shoot weight and shoot length was noted by using as protective and curative measures (Table VI). All the treatments were statistically different from each other. Minimum root weight (1.86 g) was observed where it was applied as protective measure. The control has maximum root weight (4.28 g). Maximum shoot weight and length was observed in protective treatment followed by curative.

Large numbers of galls were observed in control (Table VII). Cadusafos as protective proved effective as compared to other treatment. Control showed highest number of females and number of J2 due to pathogenic effect of nematodes. Protective application of cadusafos showed lowest number of females and minimum number of J2 as compared to other treatment after 60 days. Minimum number of egg masses (41.4) was observed in protective treatment followed by curative treatment (47.2). Maximum number of J2 was found in control plant.

DISCUSSION

Cadusafos (Rugby ®) is a broad spectrum nematicide. It controls all nematodes and particularly the most dangerous genera widespread in our region *Meloidogyne* and *Globodera*. Studies on the eggs of *Globodera* demonstrated that this nematicide has ovicidal activity.

J2 mortality recorded in 1% concentration of Cadusafos (Rugby ®) would be effect of different concentrations of nematicide on the oxygen up take of J2 of *M. javanica* (Nordmeyer *et al.*, 1982; Nordmeyer and Dickson, 1984) and the toxicity of cadusafos (Nordmeyer and Dickson, 1989). Reduction of nematode population was due to the activity of cadusafos (Rugby ®) by contact and through ingestion, when the nematode pierce the external cell layers of the roots which are exposed to the soil (Putter *et al.*, 1981; Jansson and Dybas, 1998; Faske and Starr, 2007; Monfort *et al.*, 2006).

Table V.- Effect of cadusafos on the invasion and development of *M. incognita* on tomato 35 days after inoculation

Treatments	Dosage	Developmental stages					
		Vermiform	Swollen	Sausage	Immature females	Adult females	Egg masses
Cadusafos	1.00%	63.0 d*	33 d	22 d	4.2 d	28.6 d	23.6 d
	0.50%	81.2c	41.2c	30.6 c	11.0 c	97.2 c	67.4 c
	0.25%	111.2 b	52.8b	41.2 b	33.2 b	222.8 b	191.8 b
Control		137.2 a	73.8 a	63.2 a	43.2 a	326.0 a	297.2 a

Table VII.- Nematode reproduction parameters treated protectively and curatively by cadusafos (1%).

Cadusafos (1%)	No. of galls	No. of females	No. of egg masses	J2/root system	J2/100 cm^3 soil
Protective	43.2 c*	54.4 c	41.4 c	3,260.2 c	801.4 c
Curative	53.2 b	66.0 b	47.2 b	3,774.2 b	931.0 b
Control	287.4 a	310.4 a	261.0a	17,121.2 a	4,308.0 a

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

Table VI.- Growth responses of tomato plant against *M. incognita* treated protectively and curatively by cadusafos (1%).

Cadusafos (1%)	Root weight (g)	Shoot weight (g)	Shoot length (cm)
Protective	1.86 c*	5.26 a	22.2 a
Curative	2.46 b	4.68 b	18.0 b
Control	3.60 a	4.28 c	12.8 c

* Mean followed by a distinct letter are significant at P = 0.05 according to LSD.

Cadusafos (Rugby ®) was observed to reduce invasion and development of nematodes at all stages. Vermiform was not observed after 17 days due to the fact that nematodes passed to the next developmental stage. The protective treatment of cadusafos was more effective than curative. Minimum number of galls, females and J2 was observed in protective application of cadusafos. Our results are in conformity with those of Hussain and Masood (1995); Krishna and Dabar (2004) and Patel *et al*. (2004).

Cadusafos (Rugby ®) has been reported to reduce the *M. arenaria* population on winter-grown oriental melon from 35 to 90 % compared with control (Kim *et al.*, 2002). Rehman *et al.* (2006) tested the Rugby-10 G, Furadan 3 G, advantage 5 G and Unihypo-3.6 G against root-knot nematode. They reported that Rugby 10 G proved the most effective with minimum decrease in plant height, minimum increase in fresh and dry root weight. They also showed that Rugby 10 G proved to be the best in reducing root knot disease and increasing plant vigor, followed by Furadan-3 G, advantage-5 G and Unihypo 3.6 G, respectively. Dubey and Trivedi (2011) also showed the similar results while testing various nematicides in the field giving Rugby 10 G most effective in minimizing the disease infection.

Primary efforts for screening and necessarily systemic reduction in root-knot severity in okra was attempted by Sivakumar *et al.* (1973, 1976), Sitaramaiah and Vishwakarma (1978) by using the different systemic chemicals *viz.*, Vegfru Foratox – 10 G, Vegfru Diafuran – 3 G and Rugby – 10 G were tried as nematicides for controlling most hazardous root-knot nematode in okra. All the three chemicals in different doses effectively reduced root galling and enhanced crop yield to varying degrees but best growth was observed with Rugby – 10 G followed by Foratox – 10 G and Vegfru-Diafuran – 3 G.

Giannakou *et al*. (2005) reported that oxamyl provided some nematode control while cadusafos unsuccessful to provide adequate nematode control, which may be credited to the inability of the nematicide to reduce nematode populations even at relatively high concentrations in soil. Similarly Radwan *et al*. (2012) also tested various chemicals against *M. incognita* and found that fosthiazate had the highest nematicidal effect with 97.52% reduction in galls and 96.45% juveniles in soil, while cadusafos was relatively least effective causing 77.51 and 86.63% reduction in galling and J2 population, respectively.

On the contrary, cadusafos suppressed *M. incognita* invasion and development on tomato. Similar results were also recorded where cadusafos was found to be superior in reducing nematode population and increased the yields over ethoprophos (Stephan *et al*., 1998; Meher *et al*., 2010).

It was accepted that the mode of action of carbamate and organophosphate (cadusafos) was reasonably certain that these compounds acted by the inhibition of acetylcholinesterase (ACHE) at cholinergic synapses in the nematode nervous system. Inhibition of ACHE was most likely explanation for the observed effect of organosphosphate and carbamate nematicides on the orientation behavior of nematodes (Wright, 1981; Opperman and Chang, 1990). These chemicals perform their action by impairing nematode neuromuscular activity, thereby, reducing their movement, invasion, feeding and consequentially the rate of development and reproduction (Nelmes *et al*., 1973). Bunt (1987) suggested that these chemicals acted against the root-knot nematode by inhibiting egg hatching, their movement and host invasion by infective juveniles and checked further development of second stage juveniles that had penetrated the roots.

The data on the effectiveness of the nematicides can be compared in relation to the doses used for each nematicide. From the combined results, it might be concluded that all nematicidal treatments significantly reduced the egg hatching, mortality, invasion and development of *M. incognita* in the plant roots due to both a nematicidal effect on the nematodes and to an inhibition of their penetration. However, cadusafos (Rugby ®) at 1% proved to be the most effective.

CONCLUSIONS

Cadusafos (Rugby ®) is effective at all the concentrations. It is also concluded that by increasing the dose of chemical the population of nematode reduces effectively. Cadusafos (Rugby ®) at the concentration of 1% was found to be most effective. So, it can be concluded that cadusafos (Rugby ®) can be successfully used for management of root-knot nematodes as protective and curative measure as it significantly minimize the nematode population at all the developmental stages of *M. incognita*. Cadusafos (Rugby ®) would probably inhibit nematode penetration into host roots.

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