

## Short Communications

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### SEASONAL VARIATIONS IN THE OVARY AND TESTIS OF *ERIPHIA VERRUCOSA* (FORSKÅL, 1775) (CRUSTACEA: DECAPODA) FROM KARABURUN, SW BLACK SEA

**Abstract.-** The present study describes the gametogenic cycle in an economically important and endangered crab species *Eriphia verrucosa* (Forskål, 1775) in Black Sea. The ovary and testis developed throughout the year and spawning occurred from late July to the end of August. The change in water temperature correlated with the reproductive cycle of *E. verrucosa*, whereas the salinity and dissolved oxygen did not show any correlation.

**Key words:** Reproductive cycle, seasonal variation in gonad.

Crabs are considered healthy food for their high quality protein and small amount of fat and carbohydrates (Skonberg and Perkins, *Fd. Chem.*, **77**: 401-404, 2002; Musaiger and Al-Rumaidh, *Int. J. Fd. Sci. Nutr.*, **56**: 231-235, 2005; Küçükgülmez et al., *Int. J. Fd. Sci. Technol.*, **41**: 1023-1026, 2006). *Eriphia verrucosa* is a commercial crab species in Black Sea in Turkey (Holthuis, *Vrais crabes. Fiches FAO d'identification des espèces pour les besoins de la peche. Mediterranée et Mer Noire. Zone de pêche 37, 1, Végétaux et invertébrés* FAO, Rome, pp. 321-367, 1987; Altinelataman and Dincer, *Lebensmittelhyg.*, **58**: 132-135, 2007), which has been fished out in such a large amount during the past years that it has now reached the level of endangered species in the Black Sea (EFT, <http://www.cevre.org.tr/Karadeniz> 2004).

Most of the studies on the Black Sea crabs are on systematics of crabs (Holthuis, *Zool. Verh.*, **47**: 1-67, 1961; Caspers, *Rapp. Comm. Int. Mer. Médit.*, **19**: 107-115, 1968; Ates, *Turkish. J. Zool.*, **23**: 115-118, 1999; Bilgin and Celik, *Firat Üniv. Fen ve Müh. Bil. Dergisi*, **16**: 337-345, 2004), whereas little

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is known about their reproduction, more so about the reproduction of *E. verrucosa*. The aim of present study is to determine seasonal variation of gonads in *E. verrucosa* in its natural environment and to correlate this development with temperature, salinity and dissolved oxygen. This could provide useful information for management of this commercially important species in the protection areas (Kennely and Watkins, *J. Crust. Biol.*, Lawrence, **14**: 146-150, 1994).

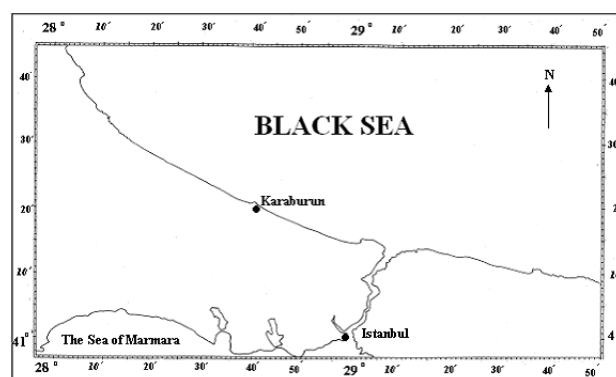


Fig. 1. Map of the investigated area.

#### Materials and methods

The crab samples were collected by using trammel net at the depth of 1 to 5 m in Karaburun located in the coast of the Western Black Sea every month during September 2004 and August 2005 (Fig. 1). The species was identified using a range of references (Bouvier, *Décapodes Marcheurs. Faune de France* (ed. P. Lechevalier et Fils), pp. 404, Paris, 1940; Zariquiey-Alvarez, *Crustáceos Decápodos Mediterráneos*. Instituto Español de Estudios Mediterráneos, pp. 181, Barcelona, 1946, *Crustáceos Decápodos Ibéricos*. Investigación Pesquera, 32: pp. 1-510, Barcelona, 1968; Demir, 1952; Holthuis, *Vrais Crabes. Fiches FAO d'identification des espèces pour les besoins de la peche. Mediterranée et Mer Noire. Zone de pêche 37, 1, Végétaux et invertébrés*. FAO, Rome, pp. 321-367, 1987). The crabs transferred to laboratory alive in sea water were killed by exposing them to a temperature of  $-20^{\circ}\text{C}$  for 15-20 minutes. The

carapace width (CW) and length (CL) of all crabs were measured with a vernier caliper at the nearest 0.01 mm. The female and the male gonads were taken out and quickly fixed in Bouin's solution for 12 hours at 20°C. The 5-6  $\mu\text{m}$  thick histological sections were cut and stained with haematoxylin and eosin (H&E) (Humason, *Animal tissue techniques*. H.W. Freeman and Company, San Francisco, 1972).

The histological sections were studied under light microscope for determining different stages of reproductive cycle. Following parameters were used for characterizing different reproductive stages. The stage 1 was designated as slightly developed stage characterized by thin, brown red ovary in colour; stage 2 designated as moderately developed stage characterized by lobed brown red ovary; stage 3 designated as developed stage is characterized by ovary with well-developed lobes brown red in colour; stage 4 designated as well developed stage by ovary with well-developed lobes and fill the anterior portion of carapace; and stage 5 designated as mature stage is characterized by ovary with well-developed lobes and fully matured eggs which fill the anterior portion of carapace. In males, maturity is established by the presence of spermatophores in the vas deferens. Similar criteria were used by Kwei (*Zool. J. Linnean Soc.*, **64**: 151-175, 1978) and Armstrong (*N. Z. J. Mar. Freshw. Res.*, **22**: 529-536, 1988) for the male *Callinectes latimanus* and by Watson (*J. Fish. Res. Bd. Canada*, **27**: 1607-1616, 1970) for the male spider crab, *Chionoecetes bairdi*.

Some ecological parameters such as salinity (‰), temperature (°C) and dissolved oxygen ( $\text{mg l}^{-1}$ ) were measured at the sampling site. The Mohr-Knudsen method (Ivanoff, *Introduction al'océanographie*. Tome I. librairie Vuibert, pp. 642, Paris, 1972) was used to measure salinity and the Winkler's method (Winkler, *Berlin Deuth. Chem. Ges.*, **21**: 2843-2855, 1888) to measure dissolved oxygen (DO).

### Results

In the present study, 142 males and 61 females were studied with CL 53-73 mm and CW 71-93 mm for males, and with CL 42-57 mm and with CW 58-80 mm for females. In sample stations, the temperature ranged between 5.5 and 25.6°C. The lowest temperature (5.5°C) was measured in

February and the highest temperature (25.6°C) in August. During this study, the salinity ranged between 16.6 ‰ and 17.9 ‰, the lowest was in February and May and the highest in August. The oxygen content of sea water in sampling stations ranged between 6.8 and 19.5  $\text{mg l}^{-1}$ . The lowest oxygen content was measured in May and the highest in June. The monthly average values of water temperature (°C), salinity (‰), and dissolved oxygen ( $\text{mg l}^{-1}$ ) are shown and the correlation between these values is given in Figure 2.

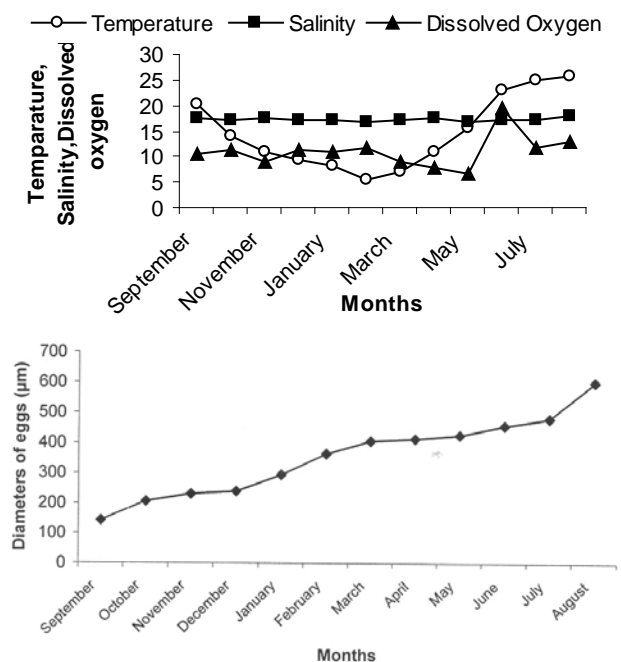


Fig. 2. The correlation between temperature (°C), salinity (‰), and dissolved oxygen ( $\text{mg l}^{-1}$ ) values with the diameter of eggs of *Eriphia verrucosa*.

### Females

Stages of development of ovaries and histological structures are shown in Figure 3. As oogenesis begins, oogonia (57  $\mu\text{m}$  in diameter) in the germinative zones of the ovary begin to produce previtellogenic oocytes (143  $\mu\text{m}$  in diameter) in September and October (Stage 1, Fig. 3 A, B). As development continues the ovary increases in width and the oocytes increase in size (231-364  $\mu\text{m}$ ) with large nuclei and visible nucleoli, during November and February (Stage 2, Fig. 3 C-F). At this stage,

some oocytes undergo early vitellogenesis and then increase in ovary volume and the oocyte is 406-457  
all oocytes pass into vitellogenic stage. There is an

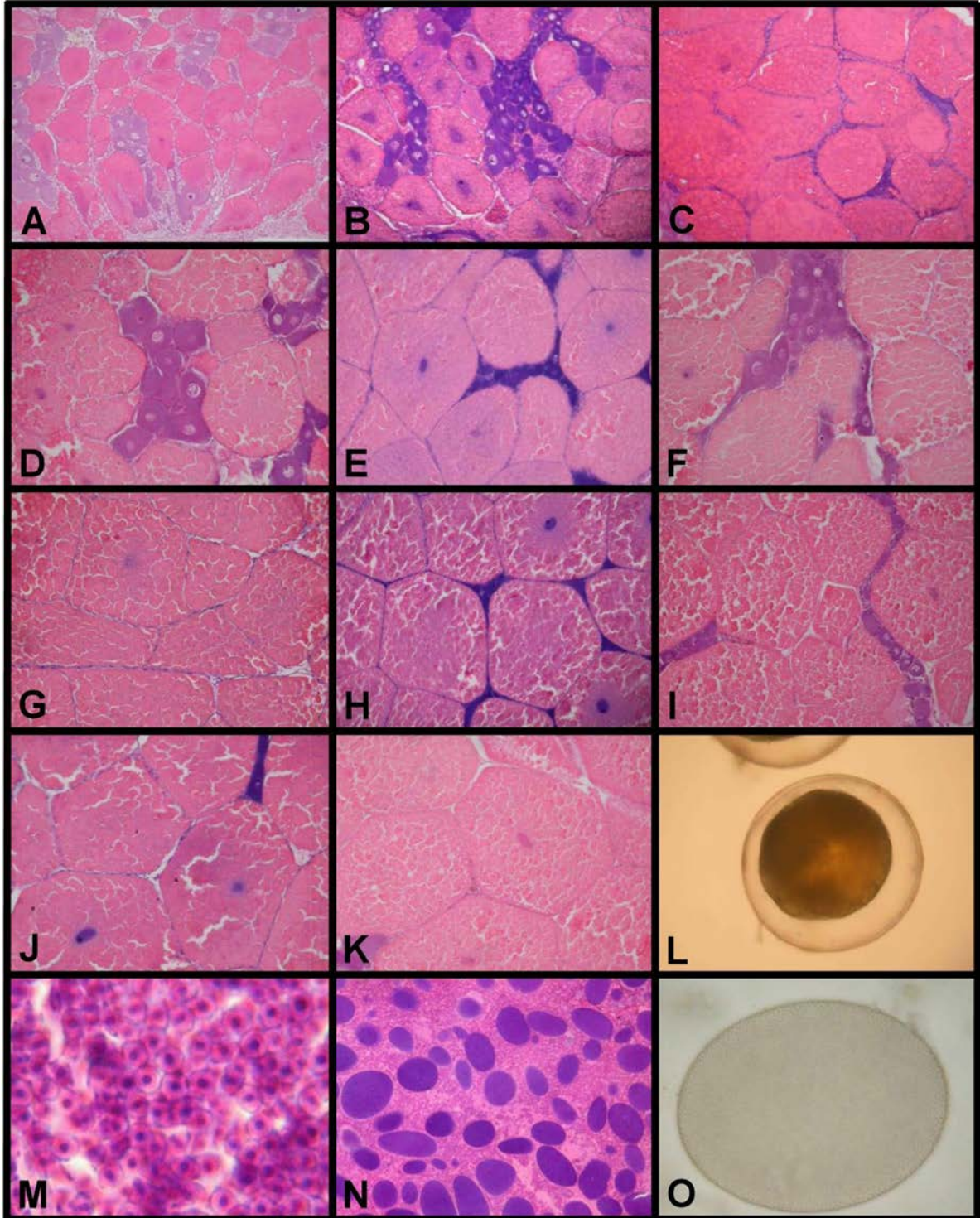


Fig. 3. Histology of ovarian (A-L) and testis (M-O) development of *Eriphia verrucosa*. A-B, Stage 1; C-F, Stage 2; G-J, Stage 3-4; K, Stage 5; L, Egg carried on the pleopods, M, Transverse section of testis; N, Transverse section of

vas deferens; O, Spermatophore has been found in the reproductive system of male *E. verrucosa*.  
Staining: haematoxylin and eosin; Magnification A-L, N, x360; M, x3600; O, X1040.

$\mu\text{m}$  in diameter between March and June (Stages 3-4, Fig. 3G-J). Most of the oocyte growth occurs between stages 3-5 content increase. Yolk content increased in the oocytes as development continues, and in histological preparations the nuclei become more obscured by yolk granules in July (Stage 5, Fig. 3K). Oocyte size reaches about 481  $\mu\text{m}$  at this stage. Eggs carried on the pleopods of the female are 601  $\mu\text{m}$  in diameter in August (Fig 3L).

#### Males

The vas deferens of mature males is always full of seminal fluid containing many spherical, fully developed spermatophores. All spermatogonial in a particular lobe of the testis are at the same stage of development. Monthly histological examination of testis of mature males showed that mature and immature spermatocytes are always present throughout 12 months. No relative increase in the size of the testis was observed at any time of the year. Figure 3M shows a typical section of testis, Figure 3N shows vas deferens, and Figure 3O shows spermatophores found in the experiments carried out in July.

#### Discussion

The structural organization of ovary and testis of *E. verrucosa* seems to follow the general pattern described for most brachyurans (Adiyodi and Subramoniam, Arthropoda-Crustacea. In: *Reproductive biology of invertebrates: Oogenesis, oviposition and oosorption* (ed. K. G. Adiyodi), pp. 443-495. John Wiley and Sons, London, 1983). Female *E. verrucosa* usually spawn from late July to the end of August in Karaburun (SW Black Sea). Pinheiro and Terceiro (*Crustaceana*, **73**: 1121-1137, 2000) verified that reproductive activity of *Arenoeus cribrarius* tended to be more intense in summer and winter, a phenomenon related to reduced temperature oscillations as found in subtropical regions. In contrast, *Portunus pelagicus* (Shields and Wood, *Mar. Ecol. Prog. Ser.*, **92**: 159-170, 1993), *Portunus spinimanus* (Santos, *Biologica reproductiva de Portunus spinimanus Latreille, 1819 (Crustacea, Branchyura, Portunidae) na região de Ubatuba (SP)*. Tese de Doutorado, UNESP,

Botucatu, São Paulo, 158p, 1994), *Callinectes danae* (Costa and Negreiros-Fransozo, *Crustaceana*, **71**: 615-627, 1998), and *Hepatus pudibundus* (Reigada and Negreiros-Fransozo, 2000) showed a continuous reproductive pattern. In the males, the spermatocytes continued to develop throughout the year and spermatophores begin to mature mostly in early July. In tropical waters, however, berried females often occur throughout the year and spawning is often continual (Shields and Wood, *Mar. Ecol. Prog. Ser.*, **92**: 159-170, 1993). Thus, the reproductive biology of *E. verrucosa* in Karaburun (SW Black Sea) differs from that in the tropics or subtropics, with spawning only occurring at certain times of the year. Such a strong seasonal pattern of spawning is closely correlated with seasonal changes in sea temperature and implies strong seasonal growth (Xiao, *Canadian J. Fish. aquat. Sci.*, **53**: 1675-1682, 1996; Xiao, *Fish. Bull.*, **97**: 690-701, 1999; Xiao and McShane, *Fish. Res.*, **37**: 66-77, 2000; Kumar *et al.*, *J. Mar. Biol. Assoc. U.K.*, **83**: 983-994, 2003).

At sampling stations, the highest dissolved oxygen concentration observed in June, when the sea temperature was 22°C. The increase of dissolved oxygen might be due to increase in phytoplankton during these months (Yılmaz *et al.*, Karadenizde birincil üretim: üretkenlikte beslenme mekanizmaları ve ışık adaptasyonu. *Birinci Ulusal Deniz Bilimleri Konferansı* (eds. Z. Uysal and I. Salihoğlu) ODTÜ, Ankara, Turkey, 2000). The occurrence of high reproductive activity correlates with the existence of rich phytoplankton that causes increase of dissolved oxygen (Litulo, *J. nat. Hist.*, **39**: 2307-2318, 2005). The salinity values did not show significant changes throughout the study effectively on reproduction of *E. verrucosa*.

Spawning of female *E. verrucosa* was determined to be clearly correlated with water temperature since in this study spawning and larval development was observed during the period when the temperature was the highest (Stead, *Proc. Linnaean Soc. New South Wales*, **4**: 746-758, 1888; Rahaman, *Proc. Indian Acad. Sci.*, **65**: 76-82, 1967; Jones, *Studies on trawled invertebrates from Moreton Bay*. M.Sc. thesis, University of



Queensland, Australia, 1970; Meagher, *Ecology of the crab Portunus pelagicus in South Western Australia*. Ph.D. thesis, University of Western Australia, Australia, 1971; Dhawan *et al.*, *Indian J. Fish.*, **21**: 164-176, 1976; Pillay and Nair, *J. Mar. Biol. Assoc. India*, **15**: 754-770, 1976). Lardies and Wehrman (*Ophelia*, **55**: 55-68, 2001) observed the similar conditions for *Beateus truncates* and Lardies and Castilla (*Mar. Biol.*, **139**: 1125-1133, 2001) for *Pinnaxudes chilensis*.

Finally, the work has major implications for managing *E. verrucosa* crab fishery. *E. verrucosa* spawn from late July usually to the end of August. Both extrusion and hatching are generally programmed so that brood release occurs at the time most appropriate for the growth of the young (Adiyodi, Reproduction and development. In: *Biology of land crab* (eds. W.W. Burggren and B.R. McMahon), pp. 139-185. Cambridge University Press, New York, 1988; Litulo, *J. nat. Hist.*, **39**: 2307-2318, 2005). Specifically, we recommend that the fishery period be managed according to the spawning period from July to the end of August when ovarian development enters stage 5 and berried females are abundant in Black Sea.

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## HELMINTH PARASITES OF PIKE (*ESOX LUCIUS* L.), IN GÖLBASI (BURSA) DAM LAKE, TURKEY

**Abstract.-** In this study, helminth parasites of pike of Golbası Dam Lake and their seasonal variation has been investigated monthly from May 2003 to April 2004. Out of a total of 114 pikes 93 were found infected with *Tetraonchus monenteron* (Monogenea), *Diplostomum spathaceum* metacercariae (Digenea) and *Raphidiscaris acus* (Nematoda). *T. monenteron* was the most common parasite

species found in the infected pike collected in all months. This species was found in 69 of 114 fish examined. Total prevalence and mean intensity were 60.5, 77.8%, respectively. The infection varied according to the seasons and reached its maximum in May (100%). The second dominant parasite, *D. spathaceum* metacercariae also occurred throughout the study period. A total of 523 parasites were found and the infection rate was the highest in September and October (100%). Total prevalence and mean intensity were 63.1, 7.2%, respectively. *R. acus* was found in 18 of the 114 examined fish. A total of 49 parasites was recorded. The total prevalence and mean intensity were 15.7 and 2.7%, respectively. The infection levels peaked in February (42.8%). Occurrence and seasonal variations of the helminth parasite species are discussed in detail.

**Key words:** *Esox lucius*; *Tetraonchus monenteron*; *Diplostomum spathaceum* metacercariae; *Raphidiscaris acus*.

Little is known about the parasite fauna of pike in Turkey. Pike from four sites have so far been examined for the existence of parasites. Among the ectoparasites of pike, *Tetraonchus monenteron* was collected in the Bursa regions, Uluabat Lake (Öztürk *et al.*, *Israel J. Zool.*, **46**: 119-130, 2000), and Dogancı Dam Lake (Aydogdu and Altunel, *Türk Parazit. Derg.*, **26**: 87-92, 2002). The endohelminth fauna was found to be richer, two species of Digenea, *Rhipiocotyle fennica* and *Diplodiscus subclavatus* were collected from Uluabat Lake (Öztürk *et al.*, *Israel J. Zool.*, **46**: 119-130, 2000), one species of Cestoda *Bathybothrium rectangulum* from Isikli Dam Lake (Denizli) (Kır and Ozan., *Türk Parazit. Derg.*, **4**: 291-294, 2005), two species of Nematoda, *Raphidascaris acus* and *Camellanus truncates* collected from freshwater of Bursa region (Uluabat and Bayramdere Lagoon) (Öztürk *et al.*, *Israel J. Zool.*, **46**: 119-130, 2000; Öztürk and Aydogdu, *Ankara Üniv.Vet. Fak. Derg. Cilt.*, **50**: Sayı: 1, 53-58, 2003) and Isikli Dam Lake (Denizli) (Kır and Ozan., *Türk Parazit. Derg.*, **4**: 291-294, 2005) and two species of Acanthocephala, *Acanthocephalus anguillae* from Uluabat Lake (Öztürk *et al.*, *Israel J. Zool.*, **46**: 119-130, 2000) and *Neoechinorhynchus rutili* from Isikli Dam Lake (Denizli) (Kır and Ozan,

*Türk Parazit. Derg.*, **4**: 291-294, 2005) in Turkey.

The present study describes the occurrence seasonal variation of helminth parasites of the pike in the Gölbaşı Dam Lake, Turkey.

#### *Material and methods*

The fish population of the Gölbaşı Dam Lake (Bursa) was sampled monthly between May 2003 and April 2004. Fish specimens collected by gill net, cast nets or creel, were transported alive to the laboratory of the Golbasi fish farm. Fish were killed by vertebral dislocation. The gills, gastrointestinal tract, liver, kidney, heart, swim bladder, gallbladder, eyes, fins and body surfaces were examined separately under a dissecting microscope for parasites. Parasites were fixed and preserved using ammonium picroglycerin according to Gussev (*Zool. Zhurnal.*, **47**: 935-936, 1968) and Fernando *et al.* (*Methods for the study of freshwater fish parasites*, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, pp. 72, 1972). The parasite specimens were identified according to the keys of Yamaguti (*Systema helminthum*, vol. I, Interscience Publishers, London, pp. 979, 1958; *Systema helminthum*, vol. III, Interscience Publishers, London, pp. 678, 1961), Markewich (*Parasitic fauna of freshwater fish of the Ukrainian S. S. R.* Izdatel'stvo Akademi Nauk Ukrainskoi SSR Kiev 1951 (English translation by Israel Program for Scientific Translation Ltd, Jerusalem, pp. 919, 1963), Bykhovskaya – Pavloskaya *et al.* (*Key to parasites of freshwater fishes of the U.S.S.R.* Izdatel'stvo Akademi Nauk SSR, Moskova, Leningrad (English translation by Israel Program for Scientific Translation, Jerusalem, pp. 919, 1962), Gussev (*Key to parasites of the freshwater fishes of the USSR. Fauna*, vol. 2, 1985), vol. 3 (ed. O.N. Bauer), Publ. House Nauka. Leningrad, 1987) and Moravec (*Parasitic nematodes of freshwater fishes of Europe*, Kluwer Academic Publishers Dordrecht, pp. 473, 1994). Prevalence (%) and mean intensity of parasites are in accordance with Bush *et al.* (*J. Parasitol.*, **83**: 575-583, 1997).

#### *Results and discussion*

Out of 114 pikes from the Gölbaşı Dam Lake, a total of three helminth species were identified *viz.*, *T. monenteron* (Monogenea) on gills, *D. spathaceum* metacercariae (Digenea) on eyes and

*Raphidascaris acus* (Nematoda) in intestines. Table I shows prevalence and mean intensity of these three helminth species in the monthly samples of *Esox lucius* in Gölbaşı Dam Lake.

The dominant helminth parasite was *T. monenteron* being the most frequent and numerous species. This species was recorded on the pike throughout the year. The infection was the highest in the June; 60.5% of all fish were infected with *T. monenteron* with a total of 5372 parasites (Table I). The prevalence peaked in spring (May) (Fig. 1). During summer and autumn, despite high mean intensity (Fig. 2), the prevalence was low (Fig. 1).

The highest mean intensity of this species was in the summer (Fig. 2). As to the infection results of this species in Turkey, Ozturk *et al.* (*Israel J. Zool.*, **46**, 119-130, 2000) conducted the first study on seasonal distribution of *T. monenteron* in this region on Pike found in Uluabat Lake. They found this species to be the dominant parasite and recorded it during every month of the year. The infection occurred the most during spring (especially April) after which the number decreased gradually till it reached its minimum level during winter season. In a similar study in the same region, Aydogdu *et al.* (*Türk Parazit. Derg.*, **26**: 87-92, 2002) recorded that 28 pikes out of 38 were infected with 223 *T. monenteron* parasites. They recorded an increase in infection from April to June with a peak in the month of June and then a decrease during fall and winter seasons. In our current study, *T. monenteron* intensity started to increase in May and reached its peak in June (total 1087 parasites) (Table I). Thus, our study supports the results of the other two studies.

The second dominant parasite *D. spathaceum* metacercariae was found in the eye lens of infected fish. Seventy two of 114 fish were infected with this parasite (63% prevalence). A total of 523 parasites were found in 72 fish. The infection was the highest in December (Table I). The prevalence of *D. spathaceum* was a bit higher than that of *T. monenteron*. It was the lowest for *R. acus*. The levels of *D. spathaceum* prevalence were observed to fluctuate, reaching a peak in autumn during September and October (Fig. 1). August, January and May are the months when the prevalence of this species reached its seasonal maximum levels (Table

I). The mean intensity levels recorded throughout the study showed that the minimum level of mean intensity was observed in autumn and its maximum level in winter (Fig. 2).

**Table 1: Prevalence, intensity and infection data of helminths recovered from monthly samples of *Esox lucius* in the Golbasi Dam Lake.**

Month	2003						2004						Total
	May.	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	
Sample size	7	10	10	10	10	10	10	10	10	7	10	10	114
<b>Infection with <i>T. monenteron</i></b>													
Prevalence (%)	100	50	60	60	30	40	80	60	60	70	50	80	60.5
Intensity range	91-	144-	57-	25-	18-	120-	12-	15-90	10-60	4-76	7-28	24-93	
	180	321	120	128	104	240	120	62.5±	35.5±	24.6±	21.6±	64±	
	120±	217.4±	90±	65.6±	63.3±	160±	43.7±	62.5±	35.5±	24.6±	21.6±	64±	
Mean intensity ± SD	31.5	74.4	30.5	46.2	43.1	54.7	37.7	27.2	19.2	31.5	10.5	26.7	77.8
Total infection	840	1087	540	394	190	640	350	375	213	123	108	512	5372
<b>Infection with <i>D. spathaceum</i></b>													
Prevalence (%)	70	30	70	80	100	100	40	70	80	28	40	40	63.1
Intensity range	2-7	1-6	5-11	3-19	2-7	1-4	5-15	6-29	4-16	7-8	2-9	4-10	
	4.2±	2.6±	7.4±	10.5±	4.5±	2.4±	10±	7.5±					
Mean intensity ± SD	1.9	2.8	2.2	5	1.5	1.1	4.3	13.8±8	8.7±4	0.7	6.2±3	7±2.9	7.2
Total infection	21	8	52	84	45	38	40	97	70	15	25	28	523
<b>Infection with <i>R. acus</i></b>													
Prevalence (%)	14	20	0	0	10	10	10	10	20	42.8	20	40	15.7
Intensity range	4	1-9			2	1	3	1	2	1	2-9	5-10	
Mean intensity ± SD	4±0	5±5.6			2±0	1±0	3±0	1±0	2±0	1±0	3±0	3.7±2.9	2.7
Total infection	4	10	0	0	2	1	3	1	4	3	6	15	49

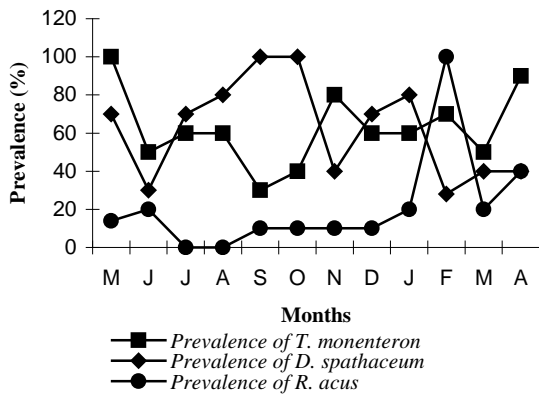


Fig. 1: Prevalence levels of the three species of helminth in the pike the Golbasi Dam Lake with respect to months.

This parasite was not recorded in Pike's helminth parasite studies conducted in Marmara Region and in Turkey until this study. Our finding is a first record of *D. spathaceum* in Pike in this region and in Turkey. On the other hand, this parasite has been recorded in different fish species in studies that determine fish parasites in this region. Aydogdu *et al.* (*Türk Parazit. Derg.*, **30**: 168-71, 2006) recorded

53 *D. spathaceum* metacercariae in 12 of 27 Bleak (*Alburnus alburnus*) species in Mustafakemalpaşa stream. In a similar study in the region, Selver *et al.*

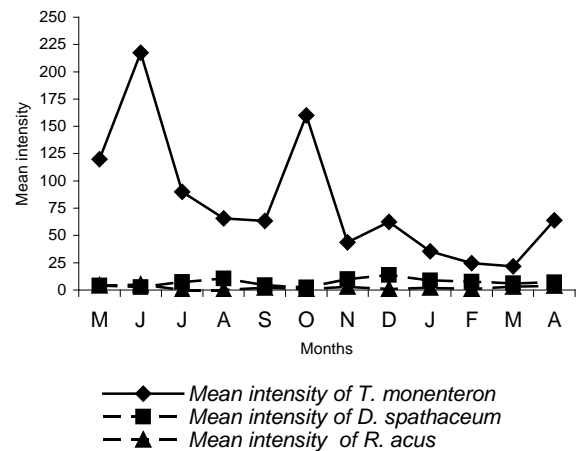


Fig. 2: Mean intensity levels of the three species of helminth in the pike the Golbasi Dam Lake with respect to season.

(*Türk Parazit. Derg.*, **30**: 151-154, 2006) recorded 365 *D. spathaceum* in 71 of 87 Rudd (*Scardinius erythrophthalmus*) (81.6% prevalence). This

parasite's infection reached its peak in the fall season in their study. (Total prevalence 95%). In our current study, we recorded 523 *D. spathaceum* in 72 of 114 Pike. The parasite's infection intensity reached its peak in the fall season (100% in September and October). Our finding about the parasite's infection intensity supports the finding of Selver *et al.* (*Türk Parazit. Derg.*, **30**: 151-154, 2006)

The third species is *R. acus*. This nematoda species was found to be a rare parasite of pike in the investigated area. A total of 49 specimens of *R. acus* were found in the intestine of host fish (Table I). Total prevalence was 15.7%, intensity 1–15 (2) nematodes per fish. The highest infection (intensity) occurred in April (Table I). For *R. acus*, while the highest prevalence was observed in February (42.8%), the lowest level was recorded in from September to January (10%) (Fig. 2). The total mean intensity of this species was 2.7 and mean intensity of *R. acus* varied according to the season and reached its highest values in summer (Fig. 2).

*R. acus* has been recorded in two previous studies by Ozturk *et al.* (*Israel J. Zool.*, **46**, 119–130, 2000) recorded 734 *R. acus* in 128 of 133 pike in their study. The parasite's infection intensity reached its minimum levels during mid-summer and its peak during the fall season. In a different study, Ozturk *et al.* (*Üniv. Vet. Fak. Derg.*, Cilt; **50**, Sayı: 1, pp. 53–58, 2003) recorded 4 *R. acus* in only 3 of 19 Pike in the months of February and March (Total prevalence 15.7%). In a study conducted at a different region in Turkey, Kir *et al.* (*Türk Parazit. Derg.*, **4**: 291-294, 2005) recorded 413 *R. acus* in 71 of 160 Pike in Isikli Dam Lake, Denizli, Turkey. The researchers recorded this parasite's infection intensity reached its peak during spring season (84.2% prevalence). In our current study, we recorded that *R. acus* reached its peak during April. A single species of Nematoda, *R. acus* was observed in 18 of the 114 fish examined. A total of 49 parasites was recorded. The total prevalence of *R. acus* was 15.7%. The parasite's prevalence reached its peak in February and April (42.8% and 40%, respectively).

To conclude, three parasites have been recorded from pike in a different locality and *D. spathaceum* metacercariae have been recorded for

the first time in pike in Turkey

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## **CENTRORHYNCHUS LÜHE, 1911 JUVENILES FROM A SNAKE (NAJA NAJA) FROM SINDH, PAKISTAN**

**Abstract.-** Surveys were carried out to determine the presence of acanthocephalan parasites in reptiles of Karachi, Sindh, Pakistan during 2004. From a single snake (*Naja naja*) more than five thousand juveniles of the genus *Centrorhynchus* (Lühe, 1911) were collected from the stomach and small intestine, which are being described.

**Key words:** *Centrorhynchus* Lühe, 1911, snake, Pakistan.

**I**n a survey of acanthocephalan parasites from snake of Pakistan more than five thousand larvae of the genus *Centrorhynchus* Lühe, 1911, were recovered from the small intestine and stomach of a single snake (*Naja naja*) collected from Karachi University Campus, Sindh, Pakistan. Thirteen specimens both male and females juveniles were stained with Mayer's carmalum, dehydrated in graded series of ethanol, cleared in clove oil and xylene and mounted permanently in Canada balsam. Photomicrographs of the acanthocephalans were taken using automatic photographic camera mounted on a research microscope Nikon (Optophot-2) in the Department of Zoology. The



specimens described in the communication are in the collection of one of the authors (A.K.).

The juvenile specimens collected had a cylindrical body, proboscis slightly swollen divided by insertion into two regions of which the anterior was entirely or partially armed with rooted hooks, while the posterior with rootless spines (Fig. 1). The hooks and spines were arranged in 20 longitudinal rows of 10-14 each. Proboscis receptacle cylindrical, neck absent, lemnisci slender longer than proboscis receptacle. Testes, cement gland unclear, similarly eggs were not observed.



Fig. 1. *Centrorhynchus* Lühke, 1911 juvenile from the snake (*Naja naja*).

Earlier *Centrorhynchus sindhensis* (Khan *et al.*, *Pakistan J. Zool.*, **34**: 309-310, 2002) has been reported from the same host, but there were only eight specimens found in the small intestine.

Presence of such large number of juveniles in the stomach and small intestine suggest that the snake is the intermediate host for this genus (Yamaguti, *Systema helminthum*. Inter Science Publishers, New York, 1963).

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## ORGANIC SOLVENT EXTRACT OF *CHENOPODIUM AMBROSIoidES* L. HAS NO ANTIBACTERIAL ACTIVITY

**Abstract:-** Hexane, chloroform and ethanol extracts of dried and pulverized *Chenopodium ambrosioides* L. were tested for their antibacterial activity at concentration of 5, 50 and 100 mg/ml against three gram positive (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) and three gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*) bacteria. None of the extracts showed any antibacterial activity against any of the tested organism. Minute irritant activity has, however, been displayed by the chloroform and ethanol extracts on applying to the ear of albino rabbits, *Oryctolagus cuniculus*.

**Key Words:** *Chenopodium ambrosioides* L. antibacterial activity, irritant activity.

*Chenopodium ambrosioides* L. (Family Chenopodiaceae) has been known for medicinal properties for many years. It is used as condiment (Argueta, *Atlas de las Plantas de la Medicina Tradicional Mexicana*, **3** Vols., 1994), against intestinal parasites and stomach ailments (Gadano *et al.*, *J. Ethnopharmacol.*, **81**: 11-16, 2002; Quinlan *et al.*, *J. Ethnopharmacol.*, **80**: 75-83, 2002), candidiasis (Rojas *et al.*, *J. Ethnopharmacol.*, **35**: 275-283, 1992), and as treatment of painful inflammatory conditions (Ibironke and Ajiboye, *Int. J. Pharmacol.*, **3**: 111-115, 2007). It also possess antioxidant activity (Kumar *et al.*, 2007).

*Chenopodium ambrosioides* is rich in monoterpenes. The whole plant especially the stem, leaves and seeds contain essential oils ascaridol, alpha-terpenes (Johnson and Croteau, *Arch. Biochem. Biophys.*, **235**: 254-266, 1984). Other

constituent are alpha-pinene, aritasone, butyric acid, d-camphor, essential oil, ferrulic acid, geraniol, 1-pinocarvone, limonene, malic acid menthadiene, methyl salicylate, myrcene, p-cymene, p-cymol, safrole, saponins, trimethyl amine, urease and vanillic acid (Klaus, *Phytomedicine*, **8**: 47-52, 2001).

The present work has been undertaken to determine the antibacterial activity of local variety of *Chenopodium ambrosioides*.

#### Materials and methods

*Chenopodium ambrosioides* was collected from the Herbarium of Government College University, Lahore.

Distilled hexane, chloroform and ethanol were used successively for the extraction of active principles from the dried and pulverized plant using Soxhlet extraction method (Furnis *et al.*, *Vogel's Textbook of practical organic chemistry*. 4<sup>th</sup> Ed, pp. 137. The English language Book Society and Longman, U.K., 1978). The extracts were concentrated on vacuum rotary evaporator (Rikakikai Co. Ltd., Tokyo) and their percentage yields were calculated.

All chemicals used were of analytical grade.

For determining antibacterial activity concentrated hexane (5.53%), chloroform (6.87%) and ethanol (6.37%) were tested against three Gram positive *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25923) and three Gram negative bacteria *viz.*, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella choleraesuis* (ATCC 13312) by using the hole plate diffusion method (Haavic and Johanssen, *J. Gen. Microbiol.*, **76**: 451, 1973). For this purpose different concentrations (5, 50 and 100 mg/ml) of these extracts were prepared in gum acacia (4.5%) solution. The solutions of antibiotics, streptomycin and ampicillin (1 mg/ml) were also prepared in the gum acacia solution. While testing the antibacterial activity hexane, chloroform, ethanol and gum acacia were also applied as blanks.

Rabbits (*Oryctolagus cuniculus*) were used to test the irritant activity of hexane, chloroform and ethanol extracts of whole plant of *Chenopodium*

*ambrosioides*. Two concentrations of extracts *viz.* 1 mg/ml and 2 mg/ml in acetone were used.

For testing the irritant activity, hair on the inner surface of rabbit ear was shaved off and divided into three portions with the help of black marker. Ten microlitre of different concentrations of each extract was applied on the three portions. The other ear was used as control where only acetone was applied in small amount. The ear was observed for redness first after every 15 min and then after every 30 min, until two of the examinations suggested that further redness would not occur. Finally ear was examined after 24 and 48 hrs to evaluate the chronic response.

The response of irritant reaction observed on rabbit ear was classified as described by Evans and Schmidt (*A Review Planta Medica*, **38**: 289-316, 1980). No reaction  $\pm$ ; doubtful reaction, +; slight redness, ++; redness between two vessels, +++; severe redness of blood vessels as well as surrounding areas; and +++++, very severe redness with exudation of fluid and marked epidermal change.

Means value and standard error were calculated. The data was presented as mean  $\pm$  S.E. (Pagano and Gauvreau, *Principles of biostatistics*, 2<sup>nd</sup> ed., pp. 38-41, Duxbury, California, U.S.A., 2000).

#### Results and discussion

None of the extracts-hexane, chloroform and ethanol exhibited any antimicrobial activity against any of the six microorganisms when compared with the standard antibiotics such as ampicillin and streptomycin. These findings do not correlate with that of Molina-Salinas (*Arch. Med. Res.*, **37**: 45-49, 2006). Irritancy testing results showed that chloroform extract displayed doubtful reaction after three hours, was prominent after twelve hours and then faded away. Ethanol extract exhibited vary prominent irritant response after three hours which lasted up to twelve hours and then faded away. The response was mild, as also reported by Vincker and Smets (*Allergy*, **56**: 1129-1136, 2001).

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## STUDIES ON THE CONTROL OF ROOT-KNOT NEMATODES AND PREVENTION OF GALL FORMATION IN TOMATO PLANTATION BY TEA DREGS

**Abstract.**- Suppression of the root galls formation and increase in length/weight of root/shoot in tomato plants (*Lycopersicon esculentum*) was observed after administration of dry tea dregs in soil at the concentration of 30 to 40 g/kg. The crop yield was also increased significantly. When the tea dregs was applied at 50 g/kg, phytotoxicity was observed. Combination of dried tea dregs as organic amendment of soil proved quite effective due to the presence of tannic acid and phenolic compound (Phytoalexin).

**Key words:** Phytotoxicity, tannic acid, tea dregs and phenolic compound.

Although there are many different species of root feeding nematodes but most important in gardens are the root-knot nematodes of *Meloidogyne* spp. They attack a wide range of plants including many common vegetables, fruit, trees and ornamental plants (Khan and Ashraf, *Pakistan J. Nematol.*, **24**: 163-169, 2006). They are difficult to control and spread easily from garden to garden in soil with field implements and shoes.

Tomato (*Lycopersicon esculentum*) is grown over an area of 2669,000 hectares with an average production of 63,986 thousand metric tones throughout the world (Chitwood, *Pest Manag. Sci.*, **59**: 748-753, 2003). In Egypt high level of infection were observed in roots of tomato field by *M. incognita*. These nematodes are highly destructive as primary partners in root-rot complexes. In

Pakistan tomato is severely attacked by root-knot nematodes. Use of nematocides for the control of root-knot nematodes is a common practice in South Asian countries (Ameen and Youssef, *Pakistan J. Nematol.*, **24**: 183-189, 2006; Whitehead, *Plant nematode control*. CAB International Wallingford, UK, pp. 384, 1998; Zureen and Khan, *Pakistan J. Nematol.*, **2**: 69-77, 1984). Due to poisonous effect of nematicides on beneficial soil microorganisms and environmental pollution, biocontrol and organic amendment are preferred (Reimann *et al.*, *Environ. Pollut.*, **115**: 239-252, 2001; Alam and Jairajpuri, *Nematode control strategies*. In: *Nematode biocontrol*. CBC Publishers & Distribution. Dehli, India, pp. 5-15, 1990; Nagdi and Yossef, *Pakistan J. Nematol.*, **22**: 157-165, 2004).

Organic and inorganic amendments of soil possess nematocidal properties *in vitro* and *in vivo* (Patel *et al.*, *Indian J. Nematol.*, December 9-10, 2005; Haseeb and Batool, *Proc. 2<sup>nd</sup> Afro Asian Nematology Symposium, Menoufiya, Egypt*, **12**: 18-22, 1994; Radwan *et al.*, *Pakistan J. Nematol.*, **25**: 395-303, 2007). Tea wastes and some oil cakes were effective for many economically important phytonematodes (Pant *et al.*, *Microflora*, **22**: 103-109, 2004; Rahu *et al.*, *Int. J. Food Microbial*, **56**: 3-12, 2000). The soil amendments with high tannin content have been found to be more effective for root-knot nematodes control (Mian and Rodriguez, *Nematropica*, **12**: 221-234, 1982).

Various plant pathogens attacking tomato plants such as root-knot (*Meloidogyne* spp.) produce a loss upto 21% in North West Frontier Province (Hussain *et al.*, *Int. Proc. Second Int. Workshop on plant Nematology*, **11**: 22-26, 1993). In this report effect of dry tea which has 15% tannins of the dry weight has been studied on the gall formation and the growth of tomato plants.

### Materials and methods

This experiments were conducted at the PCSIR Green House during October to December 2004. Dry dregs of tea (*Camellia sinensis* L.) were mixed with autoclaved sand loam soil @ 5, 10, 20, 30, 40 and 50 g/kg. The controls did not contain dregs of tea. Soil samples were transferred in clay earthen pots (30cm diam.). The pots were arranged in the green house bench in completely randomized

design at 28±5°C for 20 days to allow decomposition of organic material. The water was applied to each pot. Each treatment was replicated four times.

After 15 days of germination, seedlings were transplanted to each pot (4 plants/pot).

A water suspension of about 3000 freshly hatched second stage juveniles of *M. incognita* were added to each pot at the time of sowing, the inoculum was added 2-3cm below the top of the pot.

Two months after inoculation the plants were carefully removed from the pots. The roots were gently washed in tap water to remove adhering soil particles. Height of shoot, length of root, weight (fresh/dry) of shoot / root were determined. The number of galls/root system were recorded. The data was subjected to analysis of variance (Steel and Torrie, *Principles and procedures of statistics*. McGraw-Hill Book Co. New York, pp. 481, 1980) and least significant differences were calculated.

#### Results and discussion

Table I shows the effect of dry tea dregs on the root gall formation by *Meloidogyne incognita* and different parameters of growth of tomato plants. The data shows that the number of root galls decreased and the plant growth increased after exposure to different concentrations of tea dregs as soil amendment. Root galls formation decreased sharply with application of 20-40g tea dregs g/kg. However, above 30g decrease of gall formation is not very significant. The growth of plant is increased at 20 and 30 g/kg tea dregs, but the growth slows down at more than 30 g/kg.

**Table I.- Effect of dry tea dregs on root gall formation by *Meloidogyne incognita* and growth of tomato plants.**

Tea dregs (g/kg soil)	Shoot length (cm)	Shoot weight (fresh / dry) (g)	Root length (cm)	Root weight (fresh/ dry) (g)	No. of galls/ plant
0	9.3	2.4/1.2	4	1.8/0.7	42
5	11.5	4.68/1.99	5.55	2.32/0.98	28
10	17.2	7.50/2.87	8	3.07/1.77	25
20	29.25	15/8.00	13.2	5.87/2.2	18
30	42.4	21.35/11.35	15	5.95/3.04	11
40	38.8	19.00/7.40	12.5	4.53/3.00	12
50	37.15	17.8/5	11.85	3.75/1.87	10

LSD (0.05)	20.39	11.19/5.48	6.22	2.43/1.3	17.31
LSD (0.01)	33.81	18.56/9.08	10.3	4.08/2.2	28.7

The suppression of gall formation can be attributed to tannic acid (diglic. acid) and phenolic compound, whereas the increased growth is due to the potassium in the tea dregs. It is already known that potassium fertilizers overcome that damaging effect of root-knot (Shaikh *et al.*, *Biosci. Res. Bull. India*, **22**: 131-134, 2004). The slower growth of plants in the presence of higher concentrations (40 and 50 g/kg) of tea dregs presumably due to decomposition of tannic acid could be phytotoxic (Alam *et al.*, *Ind. J. Nematol.*, **9**: 146-148, 1979).

Organic amendments with high phenolic compound or tannin contents such as resberry cones, bark of oaks, chestnut, hemlock, sumae and berry are rich in tannis (Moyer *et al.*, *J. Agric. Food Chem.*, **50**: 519-525, 2002; Hakkinen and Torronen, *Food Res. Int.*, **33**: 517-524, 2000). There are several tannin bodies in tea, some are very closely related and belonging to the catechin group of polyphenols. The ash of the tea contain about 50% potash. Major function of tannin is against attack by bacteria and fungi. Most of the phytoalkins are phenolic compound (Darvill and Albersheim, *Annu. Rev. Plant Physiol.*, **35**: 243-275, 1984).

The results of present study indicates the soil amendments with tea dregs have potential to control root-knot nematodes. Non chemical method for nematode control can be developed which is free from hazards (Tiyagi *et al.*, *Int. Pest Contr.*, **32**: 70-71, 1990; Noling and Becker, *J. Nematol.*, **26**: 573-586, 1994; Zarina *et al.*, *Pakistan J. Nematol.*, **24**: 199-204, 2006). There is need to work out the cost benefit ratio on the use of tea dregs as organic amendment under field condition.

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