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ON THE OCCURRENCE OF SOME PLANT-PARASITIC NEMATODES WITH SPECIAL REFERENCE TO NEW HOSTS IN WEST PAKISTAN

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During the course of population studies several species of plant-parasitic nematodes were collected in Karachi and adjacent areas from around the roots of various plants of economic importance. Some of these forms are new records for West Pakistan and some host records are new. In the earlier studies from this area, Brown[1] and Kafi[2] restricted the identification of most of the nematodes they found to generic level only. In the present paper, attempt has been made to identify the nematodes to specific level. Presently ten namatode species found in Karachi and its suburban areas, identified by help of Dr. M.R. Siddiqi of the Commonwealth Institute of Helminthology, England, are reported. Four species are new records for West Pakistan.

Materials and Methods

Soil about the roots of plants was collected in polythene bags. After extraction, the nematodes were relaxed in hot water, fixed in F.A. 4:10, processed through lactophenol and finally mounted in dehydrated glycerine[3] for examination under the microscope.

Results and Discussion

The following nematodes were found:

Helictoylenchus mulicinctus (*Cobb*, 1893) *Golden*, 1956.

Only females and larvae were collected in large numbers from around the roots of *Musa sapientum* in Malir (Karachi). The species is a new record from West Pakistan. Other species of this genus reported earlier from West Pakistan include *H. dihystera* by Kafi[2] in Karachi from about the roots of *Cynodon dactylon* and *Helicotylenchus* sp. from Lyallpur in association with *Citrus* sp., by Akhtar and Hussain.[4]

H. indicus *Siddiqi*, 1963

Only females and larvae were collected in large numbers from about the roots of *Achras zapota* in Malir. This association of the nematode and plant has not be reported elsewhere.

Hemicriconemoides mangiferae *Siddiqi*, 1961

Specimens consisting mostly of females and larvae were collected in large number from around the roots of *Achras zapota*, *Mimusops hexandra*, *Mangifera indica*, *Cocos nucifera*, *Psidium guajava*, *Saccharum officinarum*, *Carica papaya* and *Citrus* sp. in Malir, Karachi and Gadap. Brown[1] reported the occurrence of *Hemicriconemoides* sp. in West Pakistan in soil samples he collected from around the roots of *Carica papaya* in Malir, but he did not identify them to specific level. Except *Carica papaya*, the remaining plants are here recorded as new hosts for West Pakistan.

Hoplolaimus columbus *Sher*, 1963

The present authors collected this nematode from around the roots of *Musa sapientum* in Malir. This species is a new record for West Pakistan. *H. tylenchiformis* has been reported by Akhtar[5] associated with *Hibiscus esculentus* in Lahore. *Hoplolaimus* spp. have been reported by Brown[1] and Akhtar[5] from several hosts including banana.

Macroposthonia sphaerocephala (*Taylor*, 1936), *De Grisse and Loof*, 1965 (Criconemoides sphaerocephala *Taylor*, 1936).

This nematode was collected from around the roots of *Musa sapientum* in Malir. The genus is reported here for the first time in West Pakistan.

Paratylenchus *Micoletzky*, 1922

Specimens consisting mostly of females and larvae collected from about the roots of *Achras zapota* in Malir belong to the *Paratylenchus curvitatus* group of Geraert.[6] This group consists of *P. curvitatus*, *P. dianthus*, *P. hamatus*, *P. nainianus*, *P. projectus* and *P. navadus*.

In West Pakistan *Paratylenchus* spp. have been recorded by Akhtar[5] from about the roots of *Chrysanthemum* sp., *Saccharum offcinarum* and *Triticum vulgare* in Lahore and by Brown[1] associated with *Saccharum officinarum* in Malir. The association of *Paratylenchus* spp. with *Achras zapota* has not been recorded previously in West Pakistan or elsewhere.

Psilenchus hilarus *Siddiqi*, 1963

Males, females and larvae were collected from about the roots of *Achras zapota* in Malir. Brown[1] and Akhtar[5] have recorded *Psilenchus* sp. from about the roots of papaya in Malir and from about the roots of *Saccharum officinarum* in Lahore respectively. Association of this species with the roots of *Achras zapota* is entirely a new record.

Rotylenchulus reniformis *Linford and Oliveira*, 1940

Young females, males and larvae were collected from the soil about the roots of *Musa sapientum* and *Mangifera indica* in Malir. Earlier *Rotylenchulus* spp. have been reported in association with *Carica papaya* and *Psidium guajava* in Malir by Brown,[1] with *Codiaeum variegatum* in Karachi by Kafi[2] and with *Mentha piperata* and *Solanum melongena* in Lahore by Akhtar.[5] These authors did not however, indentify their material to specific level. Banana and mango are new hosts for West Pakistan.

Tylenchulus semipenetrans *Cobb*, 1913

Only larvae were collected from soil about the roots of *Citrus* sp. in Malir. Association of this nematode with *Citrus* sp. has been reported by Brown[1] in Multan and by Akhtar and Hussain[4] in Lyallpur. Brown, however, did not find this nematode in Karachi.

Xiphinema americanum *Cobb*, 1913

Specimens were collected from about the roots of *Achras zapota* and *Mangifera indica* in Malir and Karachi respectively. Akhtar[5] reported this species associated with *Agrostis* sp. *Avena sativa*, *Chrysanthemum* sp., *Saccharum officinarum* and *Triticum vulgare* in Lahore, and Akhtar and Hussain[4] with *Citrus* sp. in Lyallpur. Other species of this genus having been recorded by Akhtar[5] from Lahore include *X. insigne*, *X. pratense* and *X. radicicola*. Presently *Mangifera indica* and *Achras zapota* are added to the host list from West Pakistan.

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A REFINED TECHNIQUE OF HORMONE APPLICATION TO PLANTS

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In growth correlation experiments, synthetic growth regulators are generally administered to the experimental plants in the form of solution, dust or lanolin paste.[1] Quantitative application of growth regulators in solution is possible and has been done by many workers. But it has always been difficult to deliver accurately a certain amount of lanolin paste to the plant.[2] In majority of such experiments a high concentration of hormones is applied to elicit a certain response in the plant. Since the application of paste is not quantitative, it is not easy to determine the amount of hormone absorbed by the plant.

The following technique is designed to make the application of hormone paste uniform and suggest some means of quantitative determination of hormone uptake by the plant.

Technique

The present technique has been used for the application of indole 3-acetic acid (IAA) but it can be used for other growth regulators after a slight modification.

Preparation of the Paste. (1) One gram IAA was dissolved in a known volume of absolute ethanol to obtain a certain concentration (here 40 mg IAA/ml of solution was used). (2) Anhydrous melted lanolin (here 10 g) was weighed in the required number (here 7) in 100-ml conical flasks; 25 ml petroleum-ether (b.p. 50–60°C) was added to each flask. (3) Aliquots of IAA ethanol solution (0, 0.5, 1, 1.5, 2, 2.5 and 3 ml) and of ethanol (3, 2.5, 2, 1.5, 1, 0.5 and 0 ml) were added to the flasks 1 through 7) respectively. (4) The flasks were stoppered, clamped on a flask shaker and shaken for 15 min. (5) Flasks were opened and transferred to an exhaust oven, set at 65°C for 1 hr to evaporate ethanol and petroleum-ether. The flasks 1 through 7 having 0, 2, 4, 6, 8, 10 and 12 mg IAA/mg lanolin respectively, were stoppered and stored in a refrigerator.

Following the protocol outlined above, paste of only 4 μg C^{14} IAA/mg of lanolin was prepared separately; other concentrations may also be prepared in the same manner.

Filling of Capsules. (1) Gelatin pharmaceutical capsules of proper size (here No. 4 were used), depending upon the plant material to be treated, were opened and stuck vertically on wooden racks lined with paraffin film. (2) Paste was melted in a water-bath maintained at 65°C. (3) Capsules were filled by a preheated dropper up to the brim with IAA paste of a certain concentration by dropping the melted paste from the side of the capsules. (4) Racks carrying the filled capsules were transferred to the freezer for 1 hr. (5) Excess frozen paste was scrapped off from the mouth of the capsule by a clean razor blade. The caps were replaced on the capsules which were then removed from the racks and stored in a refrigerator. (6) A few batches of empty and filled capsules were weighed. The average weight of the former was subtracted from the latter, to find the amount of paste in each capsule. The product of the weight of the paste and concentration gave the amount of IAA in each capsule.

The amount of radioactivity present in the paste was determined by placing a few opened capsules in separate glass vial containing 16 ml scintillation fluid[3] where the toluene dissolved the lanolin paste, thus dispersing radioactive IAA. The samples were

counted in a liquid scintillation counter and DPM/μg C^{14} IAA/mg lanolin and the total radioactivity per capsule was determined.

Treatment. The linear plant organ, for example, the stump or the leaf petiole may be inserted in the capsule. If the plant organ to be treated is flat, the paste from the capsule may be slightly squeezed out and directly applied on the surface of the organ.

Determination of IAA Absorption. The amount of non-labelled IAA in the applied capsule can be determined spectroscopically,[4,5] after extracting IAA from the paste. The amount of radioactive IAA in the applied capsule can be determined by counting the samples in a scintillation counter. In both cases, the paste sticking to the treated organ must be washed off with petroleum–ether or scintillation fluid. The washings should be added to the paste solution of the used capsules. The amount of IAA absorbed will give the difference between the IAA present in the capsule before and after application.

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AMINO ACID COMPOSITION AND NUTRITIVE VALUE OF ARHAR (CAJANUS INDICUS) GROWN IN PESHAWAR REGION

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Arhar was first analysed for its amino acid contents by Jaff.[6] He found that methionine and tryptophan were the limiting amino acids. Tawde and Coma[12] reported that arhar meal and its globulin fractions were deficient in methionine, histidine and cystine. Esh and Soms determined the nutritive value of arhar and found that it contains 23.5% crude protein, 1.8% fat and 2.8% ash. The biological value and coefficient of true digestibility reported by them were 61.0 and 84.9% respectively. They also found that arhar meal fed in the raw state resulted in negative protein efficiency ratio (P.E.R.) while autoclaved sample gave a P.E.R. of 1.3.

In Pakistan, arhar is grown as subordinate crop alongwith sugarcane millet and cotton. It is used as food for human consumbtion and is available at a much cheaper rate than most of the pulses. Detailed analytical work on 'arhar' has not been undertaken in Pakistan. The present studies were designed to determine the amino acid composition and nutritive value of arhar grown in Peshawar region.

Experimental

Material

The seeds of arhar (*Cajanus indicus*), grown locally were collected and were ground in a micro sample mill, using a screen with openings of 1 mm dia and stored in tightly stoppered bottles.

Methods

Proximate Analysis. Samples were analysed for moisture, ash, ether extract, crude fibre and crude protein contents (Table 1) according to the standard methods of A.O.A.C.[1]

Preparation of Protein Hydrolysate. Arhar meal (25 mg) was sealed in test tubes, containing 5 ml 6N HCl each. The tubes were heated in the oven at 110°C for 24 hr. Tubes were opened and contents were filtered. Hydrochloric acid was removed by evaporation to dryness under vaccum over boiling water-bath. The dry hydrolysate was then dissolved in 10% isopropanol.

Amino Acid Composition. The hydrolysate was analysed for its amino acid contents by one dimensional buffered filter-paper chromatography, developed by Khan and Baker.[8] Four solvent systems were prepared by mixing various proportions of the solvent and buffered solutions. The spots of amino acids developed with ninhydrin reagent, were eluted in alcohol and their absorption was measured by photoelectric colorimeter. Proline spot was obtained with isatin reagent.

Nutritive Value. For the determination of net protein utilization (NPU) the method introduced by Miller and Bender was followed.[10] Two groups of young rats, four in each group were fed on test and non-protein diets respectively. After ten days, the rats were killed with chloroform and weighed before drying. Incisions were made into the bodies. The carcass were again weighed in the dry state. From the water content, the total body nitrogen was calculated according to Bender and Miller formula.[3]

Digestibility. The feces for the two groups of rats were collected daily in separate vessels. The total amount of feces after one week was dried at 105°C

TABLE 1. PROXIMATE ANALYSIS OF ARHAR (*Cajanus indicus*).

Sample	Moisture (%)	Ash (%)	Ether extract (%)	Crude fibre (%)	Crude protein (%)	N.F.E. (%)
Arhar	10·2	2·94	1·73	2·83	21·44	60·82

Results represent average of 4 determinations.

for 20 hr in electric oven, powdered and its nitrogen contents determined. Two samples of feces were analysed for each group of rats.

Biological Value. The biological value was calculated by dividing the NPU by true digestibility and multiplying with one hundred.

Results and Discussion

Proximate composition of arhar is shown in Table 1. The results obtained show that arhar contains 21.4% crude protein. Esh and Som,[5] Tawde and Giri[11] reported 23.5% and 23.8% respectively. Slight deviation is also noted in the value of amino acids determined in the present investigation, from those given by Vijayaraghavan[13] and Chatterjee *et al.*[4] This may be attributed to different varieties, method of analysis, climatic and soil conditions. The amino acid composition of arhar (g/100 g) found are: alanine (3.97%), arginine (5.61%), aspartic acid (11.12%), glutamic acid (16.3%), glycine (3.03%), histidine (2.27%), isoleucine (6.63%), leucine (8.36%), lysine (7.05%), methionine (0.88%), phenylalanine (8.41%), serine (5.24%), threonine (4.06%), proline (5.18%), tyrosine (3.45%) and valine (5.46%). Histidine, which has been reported in literature to be absent in arhar protein[2] was found present up to 2.27%. Arhar was found to be relatively deficient in methionine in agreement with the results given in literature.[4,13]

On comparing the amino acid composition of arhar with other pulses reported by Khan and Baker,[7] it is noted that arhar has a higher content of lysine than mung and lobia, but is lower in histidine and leucine. Moreover, it is observed that arhar has a higher concentration of phenylalanine than mung, mash, masur and lobia.

The mean NPU of the two trials is 46.0% which is superior to dried yeast (43.3%), wheat gluten (37.0%) and rice gluten (36.0%) as reported by Miller and Bender.[10]

The biological value and true digestibility were 53.7 and 85.8% respectively, in fair agreement with those reported by other workers.[5,9]

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