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Effects of of Habitats and Pesticides on Aerobic Capacity and Survival of Soil Fauna

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Objective Faunal health is largely dependent on their soil environment and available litter quality. So the effects of different soil habitats and pesticides on citrate synthase (CS) activity of soil fauna and its population were studied. **Methods** The soil animals were collected from different pedoecosystems for habitat study. Whereas *Vigna radiata* based system was selected for pesticidal observations. The field was divided into five equal plots for control and treatment of γ-BHC, quinalphos, carbaryl and cypermethrin. Soil fauna was collected by quadrat method and extracted by Tullgren funnel. Individuals of a species having similar sizes were collected for the estimation of CS activity. They were homogenized and fractions were obtained by differential centrifugation. The activity of CS was assayed spectrophotometrically. **Results** Citrate synthase (CS) activity of beetle (*Rasphytus fregi*), woodlouse (*Porcellio laevis*) and centipede (*Scolopendra morsitans*) varied significantly with respect to changes in different soil habitats. Though the CS activity of *R. fregi, P. laevis*, and *S. morsitans* differed among themselves but the highest activity of CS in these animals was in *V. radiata* and lowest in *A. nilotica* based pedoecosystem. The aerobic capacity of centipede was maximum followed by woodlouse and beetle. The treatment of γ-BHC, quinalphos, carbaryl and cypermethrin significantly reduced the CS activity of these animals. γ-BHC showed maximum reduction in CS activity indicating highly toxic effect of organochlorine on aerobic metabolism of soil fauna. However, minimum reduction was observed in response to carbaryl (in beetle) or cypermethrin (in woodlouse/centipede) leading to impairment of aerobic capacity. The differences in pesticide effects might be assigned to the differences in chemical nature of pesticides and their interactions with below-ground fauna. Treatment of γ-BHC and quinalphos reduced the population of Acari, Coleoptera, Collembola, other arthropods as well as total soil fauna. Acari was least affected by γ-BHC and maximally affected (72%) in response to quinalphos. The effect of γ-BHC was fairly similar on Coleoptera, Collembola, other arthropod and total soil fauna suggesting almost similar sensitivity to this pesticide. Likewise, quinalphos was similarly effective on Collembola and other soil arthropods. Application of carbaryl decreased Acari and Coleoptera population but increased Collembola, other arthropods and total faunal populations. However, application of cypermethrin significantly reduced the population of Acari, Coleoptera, Collembola and total soil fauna and increased the population of other soil arthropods. In both the cases, acarine population was least affected. **Conclusion** The observations show the habitat-specific variation in aerobic capacity of soil fauna. However, pesticide-dependent loss in population might be due to impairment of aerobic capacity of soil inhabiting animals in desert.

Key words: Citrate synthase; Habitat; Pesticide; Soil fauna and desert

INTRODUCTION

Recently, ecophysiological studies of invertebrates have become a matter of great attraction to modern zoologists. Temperature-associated transition from aerobic to anaerobic metabolism has been suggested for invertebrates[1-4]. Pörtner[5] reviewed climatic variations and the physiological basis of temperature-dependent biogeography in animals particularly in invertebrates. Enzymes from cold-living ectotherms often function more effectively at lower temperatures than homologus enzymes from warm-living ectotherm. This suggests that many enzymes are constrained to function more effectively over a fairly narrow range of temperatures. Virtually, an effective physiological response to temperature variation may be especially important for small insects. Functional and physiological consequences of genetic variation at phosphoglucose isomerase enzyme has been studied in the beetle, *Chrysomela aeneicollis*[6].

The food assimilation in invertebrates depends on species, type of food and the rate of food intake. In most litter feeding macroarthropod species, not more than 5%-10% of the ingested food is assimilated. The assimilated food is partly respired and partly fixed in body tissue as growth. In small terrestrial animals, it is assumed that respiration is equivalent to

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75%-90% of assimilation leaving only 10%-25% for growth[7]. Thompson and Edwards[8] discussed the effects of pesticides on non-target animals. Brown[9] reviewed the effects of insecticides, herbicides and fungicides on soil invertebrates. The pesticide-dependent population reduction has been documented[10-12]. Industrial pollution has now emerged as a problem of such concern which is primarily directed towards immediate effects of toxic substances on faunae. The soil ecosystem is one such component harboring many beneficial faunal groups who play an immense role in maintaining the fertility of the soil and act as a soil bioindicator[13]. Some soil inhabiting animals show higher respiratory metabolism and ammonia and urea excretion in pasture treated with carbaryl in normal agricultural dose[14]. Bharati and Subba Rao[15] observed that the sublethal concentration of phosphomidon, monocroptophos and dichlorvos reduce carbohydrate and glycogen content and increase phosphorylase 'a' and 'b' activity in muscle and blood of earthworm, *Lampito mauritti* (Kingberg). Fenitrothion produces similar effects on carbohydrate metabolism of body wall muscle of *Octochaetona pattoni*[16]. Reddy *et al*.[17] have noted an increase in the blood sugar level under fenitrothion treatment. Although persistence of insecticides in soil and their effects on density of non-target organisms is minimum at a normal agricultural dose, the effect is obvious at population metabolism with changes in physiological and biochemical responses. Physiological correlates and ecological consequences in decapod crustaceans have been documented[18]. The studies on metabolism of soil fauna is sparse. Since faunal health is largely dependent on their soil environment and available litter quality, the changes in habitats (normal and pesticide contaminated) might influence aerobic capacity and, in turn, animal population. Therefore, the effects of different soil habitats and pesticides on CS activity of some selected soil fauna (*Rasphytes fregi*, *Porcellio laevis*, *Scolopendra morsitans*) and faunal population were studied to reflect the changes in aerobic capacity leading to population reduction.

MATERIALS AND METHODS

Animal and Pesticide Treatment

R. fregi, P. laevis, and *S. morsitans* were collected from *Vigna radiata*, *Zizyphus maurtiana, Prosopis cineraria, Accacia nilotica*, and *Salvadora persica* based soil systems for habitat study. However, *Vigna radiata* based pedoecosystem was selected for pesticidal study. This field was divided into five equal plots for control (without pesticide) and treatment of γ-BHC, quinolphos, carbaryl and cypermethrin. Each plot was subdivided into three equal parts (replications). The pesticides were applied on growing crop. The recommended doses of commercial grade γ-BHC (800 mL/acre), quinolphos (700 mL/acre), carbaryl (175 mL/acre) and cypermethrin (110 mL/acre) were sprayed in the field. *R. fregi, P. laevis*, and *S. morsitans* were collected from these plots after one day of pesticide treatment for enzymatic study. The population of different groups of soil fauna was also recorded after 15 days of pesticide application.

Faunal Collection and Extraction

Fauna were collected by quadrat method for population study, mean data of five quadrats (each 25×25 cm size) of a plot were used for converting faunal population into per square meter. The collected soil samples were sent to the laboratory in poly bags having a label indicating the date of collection, habitat, place name, vegetation, etc. These samples were processed for extraction of soil fauna by employing Tullgren funnel. A 60 watt bulb was used in Tullgren funnel. Light intensity of bulb was regulated by a rheostat control. Fauna were allowed to move down into the funnel for 24 hours. The fauna were collected in vials containing 70% alcohol and the vials were numbered for the record of soil faunal collection sites. The different groups of soil arthropods were sorted out by naked eye and under stereoscopic microscope.

Enzyme Extraction

Individuals of a species having a similar size and appearance were collected for the study of aerobic enzyme (citrate synthase). Animals were sacrificed, washed in saline, cleaned and weighed. A 10% homogenate (w/v) was prepared in Tris-HCl buffer (0.1 mol/L, pH 7.5) containing sucrose (0.25 mol/L) using Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenates were centrifuged at 700×g for 15 min in a high speed refrigerated centrifuge. The supernatant was decanted and centrifuged at 12 000×g for 20 min to obtain the mitochondrial pellets. The mitochondrial pellets were washed twice in 0.1 mol/L Tris-HCl buffer (pH 7.5) to avoid cytoplasmic contamination and each washing was followed by a centrifugation at 12 100×g for 15 min. The pellets were resuspended in the above buffer and homogenized at a high speed. The homogenized suspension was recentrifuged at 21 000×g for 15 min to remove particulate matter. The resulting supernatant was taken as a mitochondrial fraction for the assay of CS. The marker enzyme,

lactate dehydrogenase (LDH), was assayed in the mitochondrial fraction to test cytoploasmic contamination. The procedure employed for subcellular fractionation and extraction of the mitochondrial enzyme was based on Foster and Moon[19].

Assay Procedure

The procedure adopted for assay of citrate synthase (CS) was that of Foster and Moon[19]. CS activity was measured in a medium containing 100mmol/L Tris-HCl (pH 8), 0.4 mmol/L oxaloacetate, 0.2 mmol/L acetyl-CoA and 0.1 mmol/L 5, 5′-dithiobis (2-nitrobenzoic) acid in 40 mmol/L sodium phosphate buffer. The total volume of the reaction mixure was 3 mL. The reaction was initiated by addition of oxaloacetate and extinction at 412 nm was monitored. The absorbance increase in the presence of supernatant, but without oxaloacetate, was first recorded. The complete reaction was then initiated by addition of oxaloacetate.

Statistical Analysis

An one-way analysis of variance (ANOVA) was performed followed by Duncan test. The level of significance was set at 0.05.

RESULTS

Habitat Effect on CS

Changes in CS activity of *R. fregi* due to variation in pedoecosystems were significant (P<0.05) except among the soil habitats of *V. radiata, Z. maurtiana,* and *P. cineraria*. The CS activity of *R. fregi* collected from *Z. maurtiana, P. cineraria* and *S. persica* based soil system was more or less equal. Similarly, the activity of CS beetle abtained from *P. cineraria, A. nilotica*, and *S. persica* did not differ among themselves (Fig. 1). Variation in CS activity of *P. laevis* due to different soil habitats was significant (P<0.05) except among *V. radiata, Z. maurtiana, P. cineraria* and *S. persica.* Changes in CS activity of *S. morsitans* due to variation in pedoecosystems was also significant (P<0.05). However, the CS activity of centipede did not differ significantly between *V. radiata, Z. maurtiana* and *P. cineraria.* Likewise, the CS activity of centipede collected from *P. cineraria, A. nilotica* and *S. persica* did not vary among themselves. Maximum activity of CS of *R. fregi*, *P. laevis*, and *S. morsitans* was observed in *V. radiata* based soil system. However, minimum CS activity was found in these animals collected from *A. nilotica*.

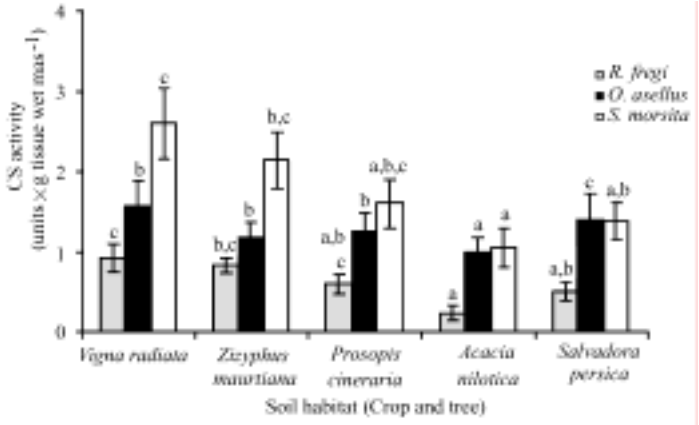

FIG. 1. Effect of crop and tree based soil habitats (pedoecosystems) on citrate synthase activity (units×g tissue wet mass^{-1}) of *Rasphytus fregi, Oniscus asellus*, and *Scolopendra morsitans*. Values are $\bar{x}\pm s$ of six determinations. Means with different letters are significantly different (P<0.05, ANOVA).

Pesticidal Effect on CS

CS activity of *R. fregi* varied significantly (P<0.001) with respect to changes in pesticide treatment except in treatment with γ-BHC and quinalphos as well as carbaryl and cypermethrin. Treatment of γ-BHC, quinalphos, carbaryl and cypermethrin reduced the CS activity of *R. fregi* by 74%, 63%, 30%, and 44%, respectively as compared to control (Fig. 2). The variation in CS activity of *P. laevis* due to changes in pesticide was significant (P<0.05) except in quinalphos, carbaryl and cypermethrin. Treatment of γ-BHC, quinalphos, carbaryl and cypermethrin decreased the CS activity of *P. laevis* by 63%, 45%, 53%, and 33%, respectively as compared to control (Fig. 2). The CS activity of *S. morsitans* varied significantly (P<0.05) with respect to changes in pesticide treatment except in treatment with γ-BHC and carbaryl as well as quinalphos and cypermethrin (P>0.05). Treatment of γ-BHC, quinalphos, carbaryl and cypermethrin reduced the CS activity of

S. morsitans by 67%, 49%, 58%, and 36%, respectively as compared to control value (Fig. 2).

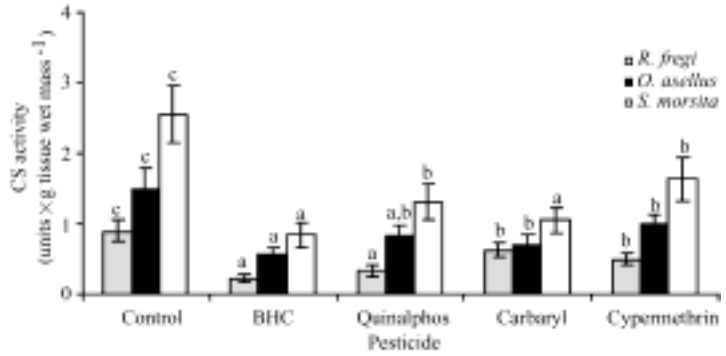

FIG. 2. Effect of different pesticides on citrate synthase activity (units×g tissue wet mass^{-1}) of *Rasphytus fregi, Oniscus asellus* and *Scolopendra morsitans* in *Vigna radiata* based soil system. Values are $\bar{x}\pm s$ of six determinations. Means with different letters are significantly different (P<0.05, ANOVA).

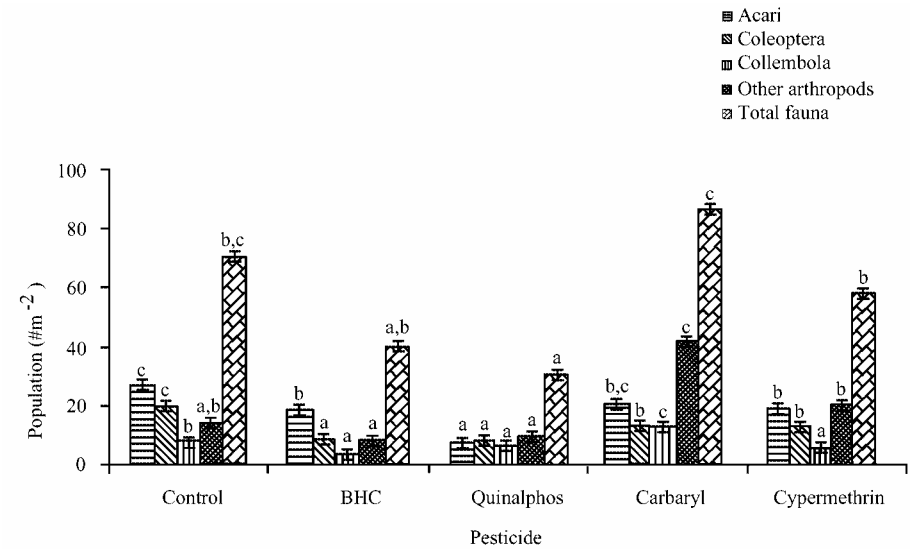

FIG. 3. Effect of different pesticides on population of various soil faunal groups. Values are $\bar{x}\pm s$ of six observations. Means with different letters are significantly different (P<0.05, ANOVA).

Pesticidal Effect on Faunal Population

Population of Acari varied significantly (P<0.001) with respect to changes in pesticide treatment except in treatment with γ-BHC, carbaryl and cypermethrin. Treatment with γ-BHC, quinalphos, carbaryl and cypermethrin decreased the population of Acari by 32%, 72%, 24%, and 30%, respectively as compared to their controls. Population of Coleoptera changed significantly (P<0.001) due to changes in different pesticides except in carbaryl and cypermethrin as well as γ-BHC and quinalphos. Treatment with γ-BHC, quinalphos, carbaryl and cypermethrin reduced the population of Coleoptera by 56%, 60%, 36%, and 35%, respectively as comp- ared to controls. Population of Collembola varied significantly (P<0.001) with respect to changes in pesticidal treatment except in treatment with γ-BHC, quinalphos and cypermethrin. Treatment with γ-BHC, quinalphos and cypermethrin decreased the population of Collembola 52%, 32%, and 40%, respectively as compared to controls. However, treatment with carbaryl increased (1.5 fold) the population of Collembola. Population of other soil arthropods changed significantly (P<0.001) due to changes in different pesticides except in γ-BHC and quinalphos. Treatment with γ-BHC and quinalphos reduced the population of other soil arthropods by 43% and 37%, respectively. Whereas the treatment with carbaryl and cypermethrin increased the population of soil arthropods by 2.9 fold and 1.4 fold, respectively as compared to controls. Population of total soil fauna also varied significantly (P<0.001) with respect to changes in different pesticides.

Treatment with γ-BHC, quinalphos and cypermethrin decreased the population of total fauna by 43%, 57%, and 18%, respectively. However, the treatment with carbaryl increased (1.3 fold) total faunal population in comparison to control values.

DISCUSSION

Variation in CS activity of beetle (*R. fregi*), woodlouse (*P. laevis*) and centipede (*S. morsitans*) due to changes in their pedoecological habitats clearly demonstrates the impact of pedoecosystem on aerobic capacity of soil fauna (Fig. 1). Not only the CS activity of beetle, woodlouse and centipede differs among themselves rather it also differs significantly in a particular species collected from some pedoecosystems. The maximum CS activity of beetle, woodlouse and centipede in *V. radiata* based pedoecosystem and minimum in *S. persica* field may be due to availability of more palatable leaf litter in the former than in the later one. The different crop and tree habitats create different types of soil environment which ultimately affect the health of inhabiting soil fauna. This difference is mainly due to variation in litter quality and availability of food for soil fauna in varied pedoecosystems. The soil animals eating more are expected to give more CS activity and hence improve their aerobic capacity. The present observation is fairly in agreement to the report of Dahlhoff and Rank[6] who described the habitat-specific differences in glucose isomerase enzyme of beetles. It appears that different crop and tree based habitats largely influence feeding of soil fauna, which reflects the changes in their aerobic capacity in different pedoecosystems. The differences in biochemical constituents of leaves might also affect CS activity in different groups (beetle, woodlouse and centipede) of animals.

The treatment with different pesticides significantly reduced (30% to 74%) the CS activity of beetle, woodlouse and centipede. This is in contrast to the report of Pradhan and Mishra[14] who showed higher respiratory metabolism in other groups of soil fauna inhabiting pasture land treated with carbaryl. Similarly, organophosphate-induced increase in phosphorylase activity of earthworm has been reported by Bharati and Subba Rao[15]. However, in the present study, γ-BHC caused maximum decrease in enzyme activity of soil fauna (Fig. 2), indicating that the organochlorine pesticide is highly toxic to aerobic metabolism. The carbamate pesticide (carbaryl) showed minimum reduction in CS activity of beetle. Whereas the least reduction in enzyme activity of woodlouse and centipede was observed in response to pyrethroid (cypermethrin). There was also no significant difference between the effects of carbamate and pyrethroid pesticides on CS activity, reflecting that carbamate and pyrethroid are least toxic to aerobic metabolism in soil animals. Therefore, it can be suggested that different pesticides reduce aerobic capacity and impair aerobic metabolism in soil inhabiting animals.

The effects (50% population reduction) of γ-BHC on Coleoptera, Collembola and other arthropods were fairly similar, indicating similar sensitivity of these faunal groups to the pesticide. Whereas Acari was less sensitive (32% population reduction) to γ-BHC. The γ-BHC-dependent decreases in populations of Acari[20-21], Coleoptera[22-23], Collembola[24] and other arthropods[25-27] support the present observations. But it differs from the report of Richter[28] who recorded an increase in total acarine population after several months of BHC treatment. In contrast to γ-BHC, quinalphos was more effective in reducing the population of Acari (72%) and Coleoptera (60%) than in populations of Collembola (32%) and other arthropods (37%). The quinalphos-induced decrease in faunal population is in agreement to the report on Acari[29], Coleoptera [29-30], Collembola[29-31-32] and other arthropods[29, 33-34]. The carbaryl-dependent decrease in the population of Acari (24%) and Coleoptera (36%) and increase in Collembola (1.5 fold) and other arthropod (2.9 fold) population agree to the earlier observations on Acari[35-37], Coleoptera[35], Collembola[35] and other arthropods[38]. However, the present findings differ from other reports about the effects of carbaryl on population of Collembola[39-40] and other arthropods[35,41]. The cypermethrin related decrease (35%) in population of Acari, Coleoptera and Collembola is in support of the reports of Shires[42] and Holopainen[43]. Similarly, cypermethrin-induced increase (1.4 fold) in population of other soil arthropods is in agreement to the report of Edwards *et al*.[30] who described cypermethrin-dependent reduction in other soil arthropods.

The differing sensitivity of different groups of soil fauna to a particular pesticide may be due to differences in structure and functions of different types of soil animals. However, the pesticide-specific effects on population of Collembola and other arthropods may be assigned to the differences in chemical nature of pesticides and their interactions with soil fauna in and outside the biological system. Perhaps it is the reason that γ-BHC and quinalphos decreased Collembola and other arthropod populations, but carbaryl and cypermethrin increased them. A number of similar observations have been documented by Reddy[11]. The pesticide-induced responses in faunal population are often climate and pedoecosystem-specific. Therefore, the present observations about the effects of pesticides on

different groups of soil fauna will be important for desert agroecosystem.

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