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〈Review〉

Collembolan Species in Environmental Studies

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Abstract : Some phenomena in which insects can demonstrate environmental changes by xenobiotics are easily and simply evaluated. On this regard, general guidelines for environmental studies using insects as biomarkers which determine the effects of such changes are suggested. Insects include three-quaters of all living animal species. This diversity comprises many closely related species which may respond sole to environmental changes by xenobiotics. Insects have been used for assessing adverse effects of environmental changes by xenobiotics, especially pesticides. Collembolan species are widely used as physiological and behavioral biomarkers for the assessment of adverse effects caused by pesticides on soil environment. This review aims to evaluate the possible use of Korean Collembolan species based upon their response to environmental changes in Korean soil mainly caused by pesticides for crop protection.(Received November 26, 2001; accepted December 26, 2001)

Key words : Collembola, ecotoxicology, biomarker, pesticides, herbicides.

1. General life history and function of Collembola

Collembola (Fig. 1) are small arthropods with adults usually 2~3 mm in length but in some species reaching 10 mm. They generally have a soft body, three pairs of legs, one pair of antenna and no wings. Many species observed are white, black or various shades of grey but others are coloured and patterned. They are furnished with setae which can be numerous or sparse, fine or thick, long or short, serrated, ciliated, clavate or smooth (Greenslade, 1994). Worldwide over 6000 Collembolan species in about 500 genera have been described. The popular name of 'springtail' has been applied because some species have a 'spring', a pair of partly loined appendages at the end of the abdomen. The spring is folded beneath the body and the free end fits into a small projection. When the spring is suddenly released, the insect leaps into the air.

Collembola are found predominantly in the soil and in the leaf litter and other decomposition habitats such as logs and dung. Certain species are found on grasses, in flowers and under the bark of trees. They are virtually ubiquitous, being found in all terrestrial biomass, including mountain tops, polar regions and deserts. Many species inhabit caves. They are common also in marine and fresh water littoral habitats and have been found even in Antarctica (Frati *et al.*, 1997).

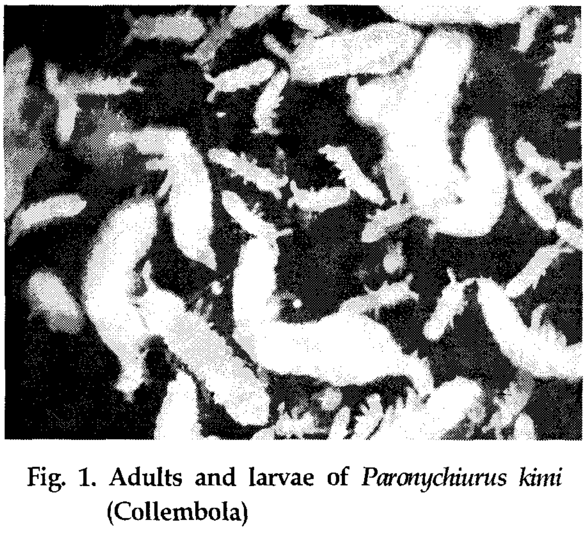

Fig. 1. Adults and larvae of *Paronychiurus kimi* (Collembola)

Collembola can reproduce by sexual reproduction or parthenogenesis. Fertilization usually takes place indirectly. The male deposits a stalked sperm sac for the female to take up. Life histories are simple since there is no metamorphosis. Spherical and pale-colored eggs are generally laid singly or in clusters in soil or

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*Corresponding author(Fax : +82-53-953-7233, E-mail : jekim@knu.ac.kr) leaf litter and hatch in a few days. The first instar closely resembles the adults in general appearance, but has fewer setae and a relatively undifferentiated cuticle. The second and third instars become progressively more similar to the adult in chaetotaxy until after five to seven instars sexually mature individuals appear. Development time from egg through to adult can take only a week in a few species, but is normally three to five weeks. Adults usually continue to grow and moult throught life (Greenslade, 1994).

Collembola feed primarily on microorganisms such as fungi, bacteria, algae and yeasts associated with the rhizosphere and with decomposing organic matter. They are generally beneficial to soils in enhancing fertility. Only a few species in the world are known to be pests. For example, *Sminthurus viridis* (L.), commonly called 'the Lucerne flea', is a quite serious pest in Australia. It feeds on clover in improved pastures in southern Australia (Bishop *et al.*, 1998). *Onychiurus pseudarmatus* (Folsom) attacks germinating seeds and roots of young plants, including vetch, wheat, celery, beans, cucumber and spinach (Getzin, 1985). Collembola are able to feed selectively on particular microorganisms. Predators of collembola include mesostigmatid mites, ants, coleoptera, hemiptera, and spiders (Greenslade, 1994).

2. Influence of Environmental factors on life cycles of Collembola

In nature there are fluctuations of food availability, humidity, soil moisture content, soil pH, and temperature throughout the year which can affect the population of Collembola on a seasonal basis. Environmental contamination of soil by a broad range of pollutants and acid rain can also affect the growth of Collembola.

2.1 Relative Humidity

Most Collembola are highly susceptible to desiccation. Many species inhabit moist soil and are very common following rain or heavy watering. The effect of relative humidity on the survival of Collembola varies according to the species. For several species studied in the laboratory relative humidity less than about 50% proved injurious to the species, whereas relative humidity greater than 75% provided good growth conditions (Ashraf, 1971). However, *Folsomia candida* (Willem) was very sensitive to small changes in relative humidity. The survival rate decreased from 100% at 99.6% relative humidity to 20% after seven days at 96.8% relative humidity at 20°C (Holmstrup, 1997). The varying responses to relative humidity changes probably result from different transpiration rates, water uptake rates and control of water loss by different parts of the body of the species such as epidermal cells or the epicuticle (Vanhoef and Witteveen, 1980). Some species such as *S. viridis* can survive hot dry summers as drought resistant eggs, which are activated by rain during the wet season (Wallace, 1968).

2.2 Temperature

Collembola from different environments can live and develop at temperatures prevailing in these environments. For a particular species changes in temperature can affect the activity and life cycle of the Collembolan. Many species grow best in the range from 10°C to 30°C in the laboratory (Ashraf, 1971), whereas an Arctic Collembolan, *Onychiurus arcticus* (Tullberg) can survive at -25°C (Worland *et al.*, 1998).

Temperature affects time from hatching to oviposition, fecundity and time and per cent of egg hatching. Egg production increases with increased temperature to about 15~20°C for many species. In *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock) it increased exponentially between 5°C and 15°C while appreciable egg development was obtained from 10~20°C (Van Staalen and Joosse, 1985). in *F. candida* oviposition decreased from 5°C to 25°C but reached a maximum at 15°C in the laboratory (Hutson, 1978). The time from oviposition to hatching declined from 16°C to 26°C, and per cent hatching was best (90 per cent) at 22~24°C but was lower both above and below this temperature (Marshall and Kevan, 1962). The time between oviposition and hatching is reduced at higher temperature. In *Hypogastrura viatica* (Tullberg) the time to hatching was 33.2 days at 8°C and 8.0 days at 26°C (Martens *et al.*, 1983).

2.3 Soil pH

Soil pH may affect the growth of Collembola. The rate of reproduction of *F. candida* was lower at a soil pH of 4.5~5.0 than at pH 6.0 (Sandifer and Hopkin, 1996). When Collembola were grown on a plaster of Paris and charcoal mixture, the optimum fecundity and hatching rate for *F. candida*, *Isotoma notabilis* (SchNffer) and *Tullbergia krausbaueri* (Borner) was pH 5.2, whereas for *P. minuta* it was pH 7.2 (Hutson, 1978).

Acid rain, which results from industrial atmospheric pollution, can cause changes in soil pH at considerable distances from the source of the pollution. The effects on forest ecosystems can be severe. Field experiments have shown that acidification of soils can have both negative and positive effects on Collembola according to the speices. In experiments carried out on pine and coniferous forest soils pH was varied by applying artificial acid rain (Buuth *et al.*, 1980), inserting acidified birch leaves (Hugvar and Kjondal, 1981), or adding crushed limestone (Hugvar, 1984). In all treatments some species increased in numbers, some declined and others remained constant. In laboratory experiments Collembola populations were monitored in pine litter, soils or sand adjusted to different pH values for periods up to 12 months (Hugvar, 1995). The numbers of several species varied with pH and changed over the period of the experiment.

2.4 Fertilizers

The effect of different fertilizers on Collembola populations has been studied. Several species were abundant in grasslands treated with nitrogen (Siepel and Van de Bund, 1988). In other experiments there was no change in the Collembola population in spring barley and grass ley fields treated with calcium nitrate (Lagerlif and AndrCn, 1991). Treatment of an oak-beech forest with P_2O_5 or K_2O fertilizers resulted in increased populations of several species (Grissen *et al.*, 1997).

2.5 Availability of food

Collembola are generally considered to be unspecialized feeders, but food preferences may differ from species to species, and food availability may be relevant to life cycles and population dynamics. Some species have been found to prefer soil-borne fungi rather than soil-borne bacteria (Harasymek and Sinha, 1974) or feed on both fungi and yeasts (McMillan, 1976). In studies where mixed fungal species were supplied one Collembola species preferred two of 15 fungi (Matic and Koledin, 1985), and several Collembola species readily consumed six fungal species whereas others were selective feeders (Chen *et al.*, 1995). Some Collembola preferred a plant pathogenic fungus to several disease control fungi (Lartey *et al.*, 1989). Some fungi were better nutrients for a particular species (Walsh and Bolger, 1993) and fungi containing high levels of nitrogen as asparagine appeared to stimulate egg production and moulting in some species (Booth and Anderson, 1979).

2.6 Elevated CO_2 level

Since elevated atmospheric CO_2 concentrations have been detected, there have been many reports describing negative and positive effects on terrestrial ecosystems and especially deleterious effects in insects (Tosi *et al.*, 1995). Some surface-inhabiting Collembola were able to tolerate CO_2 concentrations in the range of 5~10% for 1 hour whereas a species from deeper soil layers could tolerate 25% CO_2 (Zinkler and Platthaus, 1996).

3. The toxicity of agrochemicals to Collembola

3.1 General methods used for the determination of the toxicity of chemical compounds to insects

There are many methods for the determination of toxicity to insects that depend on the types of insects and the toxic compounds to be assessed. In topical application, the insecticide is applied directly to the insects (Barnes and Ware, 1965; Helson and Sundaram, 1993). A potter spray tower can also be used to examine the effect of direct contact of insecticides on insects (Harris and Mazurek, 1964; Thompson and Gore, 1972). In the filter paper assay method (FPA) the insecticide in a volatile solvent is placed on a filter paper, the solvent evaporated, and insects confined on the paper (Scopes and Lichtenstein, 1967; Frampton, 1988). An insecticide in aqueous solution can be sprayed onto the surface of a plaster of Paris and charcoal mixture and insects placed on the mixture (Fabian and Paterson, 1994). Solutions of heavy metals can be sprayed over an agar plate, excess water dried off in a desiccator, and insects placed on the agar plate. Herbicide toxicity to Collembola has been determined by incorporating the herbicide into agar which was poured onto soil (Eijsackers, 1978). Toxicity of insecticides to grain storage pest and other insects (Collins and Wilson, 1987; Helson and Sundaram, 1993) and heavy metals to Collembola (Joosse and Verhoef, 1983) can be determined by inclusion of the toxic materials in food fed to insects.

For organisms which live in soil, exposure to chemicals in soil has been used in toxicity assessment and the concentrations of toxic chemicals investigated have been defined as per cent or ppm of total soil. Different soils influence toxicity determination because the behaviour and availability of toxic compounds depend on the characteristics of the soil. Compounds may appear to be more toxic (lower LD_{50} values) with increases in soil moisture content and soil temperatures; in dry soils toxic compounds may be more strongly adsorbed to soil particles than in wet soils; in both wet and dry soils toxicity is reduced (higher LD_{50} values) in soils containing high levels of organic matter (Harris, 1966; Harris, 1971; Achick *et al.*, 1989). High temperatures may increase the volatility of toxic compounds and decrease their adsorption to the soil, resulting in apparent reduced toxicity (Harris, 1971; Thompson, 1973).

The importance of the soil liquid phase (pore water) in toxicity assessment has recently been recognized because it is a major site of exposure for organic chemicals and pesticides for several soil-inhabiting animals (Lord *et al.*, 1980; Smit and Van Gestel, 1998). The relative toxicity of chemical compounds to soil fauna is similar, whether the assays are carried out in natural or surrogate pore some advantages. It is easy to set up a range of chemical concentrations; the test animals can be clearly seen in water; and sometimes difficult and time consuming procedures for extractions to the method because of the low solubility of some compounds (Ronday and Houx, 1996).

3.2 Sites of action of toxic chemicals and detoxification mechanisms

Toxic chemicals can affect insects at a variety of sites, including components of the nervous system such as acetylcholinesterase (AChE), the acetylcholine receptor site and ion channels. Other toxic compounds can affect the digestive system and disrupt insect feeding patterns. Inhibition of reproductive systems can also be a mechanism for toxicity.

Insects can become resistant to toxic chemicals by a range of mechanisms. These include behavioral, physiological and biochemical modifications. Some insects are able to detect a particular compound more readily than the overall population and may therefore avoid direct exposure. There are many example of differential absorption of chemicals into resistant and susceptible insects which can confer resistance (Little *et al.*, 1989). Increased lipid content can confer resistance to lipophilic compounds such as organochlorines. The larvae of khapra beetle, Trogoderma granarium (Everts) (which have three times more lipid content than adults) were much more tolerant to DDT than adult insects (Gupta *et al.*, 1971). Increased excretion of compounds may contribute resistance (Little *et al.*, 1989).

Biochemical mechanisms of resistance include esterase activity, glutathione *S*-transferase (GST) activity and cytochrome P450-dependent monooxygenase. Esterases may act by direct hydrolysis of an insecticide (Motoyama *et al*, 1984) or by irreversibly binding the insecticide and removing it from the system (Suzuki *et al.*, 1993). There are many examples of the presence of increased levels of GST in resistant insects (Balabaskaran *et al.*, 1989; Reidy *et al.*, 1990). These enzymes are able to form excretion is facilitated. In many insects elevated levels of cytochrome P450-dependent monooxygenase are correlated with resistance to insecticides (Yu and Terriere, 1979; Scott and Georghiou, 1986; Cuany *et al.*, 1990; Kotze, 1993).

The organochlorine compounds are persistent in nature because it is difficult to degrade the molecules and remove the chlorine atoms. However, the detoxification of DDT or lindane in susceptible and resistant flies has been assissted by conjugation with glutathione catalyzed by GST and dechlorinase (Motoyama and Dauterman, 1975; Tanaka *et al.*, 1976). Clark and Sharman (1984) showed that a purified dehydrochlorinase which catalyzed DDT dehydrochlorination in flies was also able to act as a GST.

3.3 Influence of pesticides on Collembola in field and laboratory studies

3.3.1 Field studies

Since the use of synthetic insecticides in agricultural production, starting in the 1940s, there has been a growing awareness of the consequences of insecticide use for the environment in general. There have been many studies on soil fauna communities other than the target organisms in agricultural lands after insecticide use. Insecticides used to control specific target species may have different effects on other insect populations in the environment which may alter the balance of the non-target organisms.

In considering the results of field studies on soil fauna populations it is important to remember that the effect of an insecticide on a particular species will vary according to the soil type and conditions. When soils were treated with DDT there was an increase in Collembola levels which was attributed to a reduction in the population of predatory mesostigmatic mites (Sheals, 1956; Edwards *et al.*, 1967; Perfect *et al.*, 1981). In pine forests the effect of DDT on Collembola varied according to the species; Sminthuridae levels decreased but Entomobrydae remained unchanged (Knight and Chesson, 1966). However, in lindane and alsrin treated soils the numbers of both Collembola and mites decreased indicating that these compounds are more toxic than DDT (Sheals, 1956; Edwards *et al.*, 1967). Collembola levels were reduced in wheat-growing soil exposed to aldrin and endosulfan, but recovered with time (Joy and Chakravorty, 1991).

Organophosphate insecticides used in crop production also affect non-target organisms such as Collembola. The use of dimethoate to control cereal aphids in winter wheat (Vickerman and Sunderland, 1977) resulted in reduced populations of the predators and Collembola, which remained at low levels. In an isofenophos-treated rye field the population of several species of Collembola decreased at varying rate (Krogh, 1991). Dimethoate and phosphamidon applied to mustard growing soils caused only a temporary reduction of the Collembola population before restoration to the original population level (Joy and Chakravorty, 1991).

In general, pyrethroids were less toxic to Collembola than organophosphates and carbamates (Wiles and Frampton, 1996). In barley-growing soils sprayed with fenvalerate and permethrin Collembola were significantly less abundant in a sandy loam than in an organic soil in which the insecticides were probably more tightly bound (Harris and Lichtenstein, 1961; Harris, 1971). Fenvalerate was more toxic to some Collembola than permethrin in both organic and sandy loam soils (Huusela-Veistola *et al.*, 1994). Nine insecticides were evaluated for control of the lucerne flea, *S. viridis*. Fenvalerte had no effect on the insect whereas carbaryl and seven oranophosphates were toxic and kept populations low for varying times (Bishop *et al.*, 1998).

Many Collembola are fungal feeders and the effect of some fungicides on the insect's food supply can affect population levels. When pyrazophos was used in barley fields Collembola numbers were reduced and remained at low levels (Frampton, 1988). The application of the carbamate fungicide, benomyl, on a rye field resulted in reduced populations of several Collembola species (Krogh, 1991). Two applications of the systemic fungicide Aktuan 11 weeks apart on hop-growing land resulted in reduction in the populations of typical fungal feeders, *Hypogastrura assimilis* (Krausbauer) and *P. minuta*, early after the first application, whereas deeper soil inhabiting species, juvenile Entomobryidae, *I. notabilis* and *Neelus minimus* (Willem), were reduced only after the second application. Some species were not affected because they can use a wider range of food (Filser, 1994).

In field studies the varying environmental conditions and mixed populations of soil fauna make it difficult to determine the direct toxicity of insecticides and fungicides to Collembola. laboratory studies have been used to clarify some of these interactions. In some methods sand and soil have been used in controlled laboratory conditions. In other assays alternative methods have been used for contact between the insecticides and the insects.

3.3.2 Laboratory studies

Several studies have been carried out using insecticides mixed with soils to assess the toxicity of a range of insecticides and the effects of different temperatures on toxicity. *F. candida* was exposed to 29 insecticides in a plainfield sand soil at 13°C or 24°C for 24 hour. Some insecticides were more toxic at 24°C than at 13°C; some were more toxic at 13°C; some equally toxic at 13°C and 24°C (Thompson and Gore, 1972; Thompson, 1973). In a simliar experiment using 17 insecticides at 21°C for 24 hour *Onychiurus justi portei* (Denis) was more tolerant than *Hypogastrura armata* (Nicolet), with *F. candida* the most susceptible (Tomilin, 1975). When *O. pseudarmatus* was exposed to 19 insecticides in silty clay-loam soil for seven days, LC_{50} values for the insecticides ranged from 1.23 ppm to over 50 ppm (Getzin, 1985). Methyl parathion was more toxic than carbaryl and endosulfan to *Cyphoderus* sp. in sandy-loam soil with LD_{50} for 24 hour incubation periods values for methyl parathion, carbaryl and endosulfan of 1.05, 2.6 and 2.65 ppm, respectively (Joy and Chakravorty, 1991). The maximum toxicity of these insecticides for *Cyphoderus* sp. and *Xenylla* sp. were obtained in pure sand soil, followed by sandy-loam soil, clay soil and organic soil. Folker-Hansen *et al.* (1996) reported the body growth of *Folsomia fimetaria* (L.) was adverssely affected in soils treated with dimethoate, but *H. assimilis* was not affected.

The filter paper assay has been used to determine the toxicity of insecticides and fungicides to Collembola. The LD_{50} values determined for *F. fimetaria* for 8 h incubation periods were carbaryl 0.2 μg, parathion 0.41 μg, lindane 0.92 μg, diazinon 1.45 μg and aldrin 18.0 μg per 24 cm^2 area of filter paper in a glass petri dish. However, no insects were killed by incubation with up to 90 μg per cm^2 of DDT for 24 hour (Scopes and Lichtenstein, 1967). The relative toxicity of lindane, aldrin and DDT determined in this assay is consistent with field assays which indicated that DDT was less toxic to Collembola than aldrin and lindane (Sheals, 1956; Edwards *et al.*, 1967). The fungicide, pyrazophos, was about 25 times more toxic to *Sminthurinus areus* (Lubbock) than other fungicides, carbendazim, propiconazole and triadimenol (Frampton, 1988).

The potter spray tower direct contact method was used to determine the toxicity of 29 insecticides to *F. candida* on incubation at 13°C or 24°C for 24 hour. Although the group of insecticides used was not ideal with those tested in plainfield sand soil, many compounds were tested in both systems. The relative toxicities were similar for the two assays. Aldrin, chlordane and DDT had no toxic effects at all at both incubation temperature (Thompson and Gore, 1972).

The toxicity of parathion and dimethoate to *F. candida* was determined using the saline water toxicity test. The EC_{50} values were 0.0025 ppm for parathion and 27 ppm for dimethoate after four days incubation. The low water solubility of parathion with dimethoate which has a much higher water solubility (Ronday and Houx, 1996). When the toxicity of parathion, carbofuran, dimethoate and oxamyl to *F. candida* was evaluated in a saline water medium for four days EC_{50} values were 0.009, 0.222, 16.2 and 27.6 ppm, respectively and for seven days 0.004, 0.095, 10.1 and 4.1 ppm, respectively (Houx *et al.*, 1996).

3.4 Influence of herbicides on Collembola in field and laboratory

Insect diversity is expected to be high when weed species occur in crop ecosystems (Altieri *et al.*, 1977), and environmental conditions are favourable such as higher relative humidities, moderate temperatures and a greater source of food for some insect species (Gray and Coats, 1983). Relatively large quantities of herbicides are used in agricultural production in comparison with other types of pesticides. Herbicide residues induce substantial changes in the habitats of soil fauna and some herbicides can directly affect these organisms. The effects of herbicides in ecosystems have received less attention than the effects of other types of pesticides.

3.4.1 Field studies

In the field studies most herbicides, except the triazines, have very little impact on Collembola populations (Rapoport and Cangioli, 1963; Christiansen *et al.*, 1989; Potter *et al.*, 1990). Populations of most species decreased after applications of atrazine (Al-Assiuty and Khalil, 1996). Simazine (Edwards, 1970) and cyanazine (Edwards, 1991) depressed Collembola populations. However, *Tullbergia granulata* (Mills) increased in grassland (Moore *et al.*, 1984) and corn fields (Mallow *et al.*, 1985) after exposure to atrazine.

3.4.2 Laboratory studies

The effect of atrazine on egg production, hatching and further development has been investigated. For two *Onychiurus* species, *Onychiurus apuanicus* (Dallai) and *Onychiurus armatus* (Tullberg) exposed to atrazine in sterile sand for 30 days, the LD_{50} values were 17.2 ppm and 20 ppm, respectively and egg production, hatching, and subsequent development and reproduction were not affected up to 10 ppm atrazine (Mola *et al.*, 1987). For *O. cinta* supplied with food containing atrazine, the LC_{50} was 244 ppm of food and egg production and development was unaffected to 40 ppm of food (Badejo and Van Staalen, 1992). For *Entomobrya musatica* (Stach) atrazine supplied in baker's yeast affected egg production and instar duration but high levels of atrazine, 2330 ppm of food did not affect hatching of the eggs produced (Al-Assiuty and Khalil, 1996).

4. Conclusions

Recently, Choi (2001) has demonstrated that most ecotoxicological studies using Collembolan species were limited to a few collembolan species, while most other species ignored. Thus, he used a common springtail, *Paronychiurus kimi* (Lee) found in paddy soil in Korea for determining the adverse effects caused by herbicides such as butachlor, hexazinone and paraquat. In Korea, the environmental studies using Collembolan species are sparse, thus the authors would suggest to use Collembolan species present in Korean soil to validate the adverse effects in soil environment and to predict the environmental hazard level of a new pesticide. Furthermore, to establish the importance of indirect effects and recovery for the validity of extrapolations from results of single species tests, there is a need to investigate such effects in multispecies mesocosms, including the range of taxonomic and trophic groups introduced at the single species level. As Collembolan species are major organisms present in soil, they can degrade pesticides to non-toxic metabolites. Therefore, it is worthwhile to study the role of Collembolan species for pesticide degradation in soil environment.

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환경연구에서의 톡톡이 이용

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요약 : 곤충류를 이용하여 외부로부터 유입된 물질로 인하여 변화된 환경에 적응하는 현상들을 쉽고 간단하게 평가할 수 있다. 이와 같은 관점에서 환경변화의 영향을 측정하는 생물학적 기준으로 곤충을 이용한 환경연구의 지침들이 제안되었다. 곤충은 전 세계 동물 종 중 75%이상을 차지하고 있으며, 이러한 생물적 다양성으로 인해 외부물질에 의하여 발생한 환경변화에 독특하게 대응하는 많은 유사종들이 존재하게 되었다. 곤충은 외부물질 중 특히 농약에 의하여 생긴 환경변화의 부차적인 영향들을 조사하는데 사용되어지고 있다. 그 중에서도 톡톡이는 토양환경오염에 대한 농약의 부차적인 영향을 조사하는데 생리학적 · 행동학적 생물기준으로 널리 사용되어지고 있다. 본 총설에서는 농작물보호를 위해 사용된 농약들의 토양오염에 대한 부차적인 영향을 조사하기 위하여 한국에 서식하는 톡톡이를 이용하는 방안을 검토하는데 그 의의를 두었다.

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