Agrichemicals as antagonist of lectin-mediated *Rhizobium*–legume symbiosis: Paradigms and prospects

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The *Rhizobium*–legume interactions have been reported to be very specific in nature. One of the major factors contributing for this specificity is the activity of plant root lectins. It has been speculated that the agrichemicals, accumulated due to extensive application to soil, may protect the *Rhizobium* recognition sites on the root surface of legumes. As a result, the biological nitrogen fixation and consequently the yield of leguminous crops will be decreased due to reduced nodulation. To test this hypothesis, the *in vitro* interactions of a representative herbicide, paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) with *Canavalis ensiformis* (Con A) lectin was investigated by fluorescence and CD analysis. This model study revealed significant conformational changes in the protein structure due to extensive binding of paraquat (PQ) with Con A. The binding isotherm exhibited at least 25 PQ binding sites on Con A lectin. The Scatchard analysis suggested a binding constant and a capacity of 4.7×10^6 l/mole and 16.7, respectively. Thus, the agrichemicals exhibiting higher affinities for lectins may interfere with the binding of *Rhizobium* species to the corresponding lectins on the root surface of legumes and exert colossal impact on the nitrogen supply and crop productivity.

PLANT lectins are the specific carbohydrate-binding proteins, constituting approximately 10% of the extractable nitrogen in the seeds of leguminous plants and have been extensively used in the study of cell surface architecture. Earlier work on lectin distribution in plant tissues as well as lectin mediated cell–cell interactions provides strong evidence for their involvement in the defence of plants against infection[1,2] and also in *Rhizobium*–legume symbiosis[3–7]. During the symbiotic biological nitrogen fixation, the bacteria of the genus *Rhizobium* living in the rhizospheric region of the leguminous plants, adhere to the legume roots and are subsequently internalized to form nitrogen-fixing nodules. The *Rhizobium*–legume interactions are specific and the specificity is achieved through the action of plant lectins[3–7]. It has been demonstrated that the lectin in beans extract could help bind the specific bacteria to the roots of *Phaseolus vulgaris*[3]. Systematic studies in this direction were subsequently

made in soybean–*Rhizobium japonicum* and clover–*Rhizobium trifolii* systems. The specific binding of the lectin to *R. japonicum* has been supported by the heptin inhibition[6] and morphological[5,8] studies. Earlier work on clover–*R. trifolii* system also provided convincing evidence for the involvement of lectin in specific root–bacterial interactions[9–13]. Furthermore, the binding of *R. trifolii* to root clover hairs, which remained unaltered by 2-deoxygalactose and D-glucose has been found to be strongly inhibited by 2-deoxyglucose[10]. This implies that the sugar, 2-deoxyglucose, presumably inhibits the attachment of *R. trifolii* to the lectin in the clover root epidermal cells. This observation gains credence from the fact that the capsular polysaccharide of the infecting bacteria, serving as the lectin receptor, also contains 2-deoxyglucose[11]. Also a crude preparation of lectin from clover seed caused agglutination of *R. trifolii*, which was inhibited by 2-deoxyglucose but not by glucose, galactose and glucosamine. The capsular polysaccharide containing 2-deoxyglucose also reacts positively with the antiserum raised against the infective *R. trifolii*. Interestingly, the lectin isolated from clover seeds has been found to be identical with the clover root hair lectin, in subunit molecular weight, immunological reactivity, and in the elution behaviour[12]. The clover root lectin also causes agglutination of *R. trifolii*, which could be specifically inhibited by 2-deoxyglucose. Using anti-serum against clover seed lectin, Dazzo, Urbano and Brill[13] demonstrated the localization of lectin at the root hair tips, serving as the binding site for the bacterial capsular polysaccharide. Interestingly, most of the published work deals with the lectin from seeds and not from root cells, which are the actual sites of nodulation. This point assumes special significance in view of the suspected tissue specificity of lectins and this issue has yet to be resolved. Moreover, the cell surface polysaccharides of specific *Rhizobium*, which are likely to serve as lectin receptors have either been ignored or characterized only qualitatively. Furthermore, the role of lectins in *Rhizobium*–legume interactions involving the major leguminous crops of India has not been thoroughly investigated. Lectins from these sources even when detected have rarely been characterized for their chemical, structural and biological properties and every tissue of these plants has not been assayed for lectin activity.

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Undoubtedly, very little is known about the extent and nature of interactions of the environmental pollutants, particularly agrichemicals with lectins, and their impact directly on the nematode–lectin interactions and *Rhizobium*–legume symbiosis.

Impact of agrichemicals on growth yield of legumes

Indeed, the legumes have a special role to play in areas where diet is poor in nutrients as they are considered to be an excellent source of high quality proteins. In India, about 24 million hectares are under pulse crop and about 6 million hectares are under groundnut and other leguminous fodder crops[14]. However, the available data indicate that the grain yield of blackgram, a popular and highly proteinaceous pulse crop, has been very low[15]. This is attributed to its cultivation on marginal and submarginal lands of low fertility, where little attention is paid to adequate fertilizers, specifically phosphorus and nitrogen. Moreover, the symbiotic nitrogen fixation may not be adequate to satisfy the nitrogen need of developing pods. A survey revealed that in about half the area, the nodulation was poor for one reason or the other, though the legumes are grown in the area for a long time. One reason for the poor crop yield could be the inhibitory effect of pesticides on root nodulation[16]. As demonstrated earlier, the application of lindane to soil at the rate 5 ppm and above inhibits nodulation and growth yield of chickpea, whereas the lower concentrations are non-toxic[17]. Similarly, Pawar and Chavan[18] observed that dimethioate and monocrotophos reduced the germination, while carbofuran, formothion and dimethioate reduced the plumule weight of green gram. Also, the treatment with malathion results in the reduced germination of *Vigna mungo*[19]. Furthermore, Tiwari and Chattoraj[20] working with three pulse crops, viz. pea, green gram and black gram demonstrated that treatment with aldrin, disulfoton and carbaryl adversely affects the plant growth at different doses. Comparative assessment of these insecticides revealed marked severity on black gram and green gram plants than the pea varieties with carbaryl treatment followed by aldrin and disulfoton, respectively.

Agrichemicals and root-knot nematodes as potential inhibitors of nodulation in legumes

The leguminous crops have also suffered greatly due to various diseases and pests. For instance, the plant parasitic nematodes, invariably found in the soil around the roots of plants, reportedly act as a limiting factor in the crop production. The effect of nematodes is variable on different important pulse crops of India, viz. gram, black gram, moth, urad, lentil, pea, cowpea, guar, french bean, soybean, lima bean and pigeon pea[21,22]. On a worldwide

basis, plant parasitic nematodes have been estimated to cause annual losses of US $ 177 millions to pigeon pea and US $ 328 million to chickpea. Interestingly, certain species of root-knot nematodes have been reported to reduce the nodulation in leguminous crops, particularly mung bean, chickpea, cowpea and green gram. The presence of sugars such as N-acetylglucosamine, galactose, N-acetyl-galactosamine, and mannose and/or glucose on the cuticle surface of plant parasitic nematodes[23–25] may play an important role in the interaction between nematodes and their hosts. It has been demonstrated that the binding of *Rhizobia* to nematode-free roots was inhibited only after pretreatment with certain sugars[21]. Studies on the interference of nematodes with soybean lectin metabolism showed the reduced binding of *Rhizobia* to *Heterodera glycines*-infected soybean roots, suppressing the nodule formation[21]. Furthermore, the root-knot nematode *M. incognita* infecting mungbean, chickpea, cowpea, wandopea and green gram, *M. hapla* infecting white clover, and *Meloidogyne* spp. infecting horsebean, lupin, clover and pea have been reported to inhibit nodulation[22,26–29]. Interrelationship between *M. incognita*, *Heterodera cajani* and *Rhizobium* sp. on cowpea (*Vigna sinensis*) has been investigated. Sharma and Sethi[27] reported that *M. incognita* and *H. cajani*, singly or in concomitant inoculum, significantly reduce the growth of cowpea; *M. incognita* reduced N-content to a greater extent than *H. cajani*. Similarly, Hussaini and Seshadri[30] reported that *M. incognita* inoculated before and after or simultaneously with *Rhizobium* caused significant decrease in plant height, fresh and dry weight of shoot and root, number of nodules on root and nitrogen content of root when compared to nematode-free plants. Presumably, the common sugars on the cuticle surface of nematodes compete for the plant lectins, resulting in reduced rhizobial-binding sites. As proposed in the model (Figure 1), relatively less *Rhizobia* may bind to the root hair surface of nematode-infected plants, resulting in decreased crop yield. The productivity of pest-infected crop could be improved with the judicious application of plant protection chemicals. However, due to ignorance and inadequate knowledge of pesticide application and toxicity, an indiscriminate use of various agrichemicals is in practice in developing countries. Such chemicals interact with the plants and associated microflora in the soil and exert detrimental effects when accumulated in the soil beyond a threshold limit. The precise mechanism of pesticide interaction with the plant system ensuing reduction of plant growth and yield is not yet clearly understood. It is speculated that certain pesticides exhibiting high affinity for plant lectins may interfere with the binding of *Rhizobium* species to lectins on the root surface of specific legumes. As a result, the interactions between the legume and *Rhizobium* from the analogous cross-inoculation groups will be inhibited. It is surmised that due to extensive application of agrichemicals in the soil, the *Rhizobium*

recognition site on the root surface of legumes could be protected. Consequently, the biological nitrogen fixation and yield of the leguminous crops will be suppressed due to reduced nitrogen supply to the plants and soil (Figure 2). Hitherto, the nature and the extent of interactions between the agrichemicals (pesticides) and the molecular components of the plant root cells and the Rhizobium species participating in root nodule formation are yet to be elucidated and warrant extensive investigations.
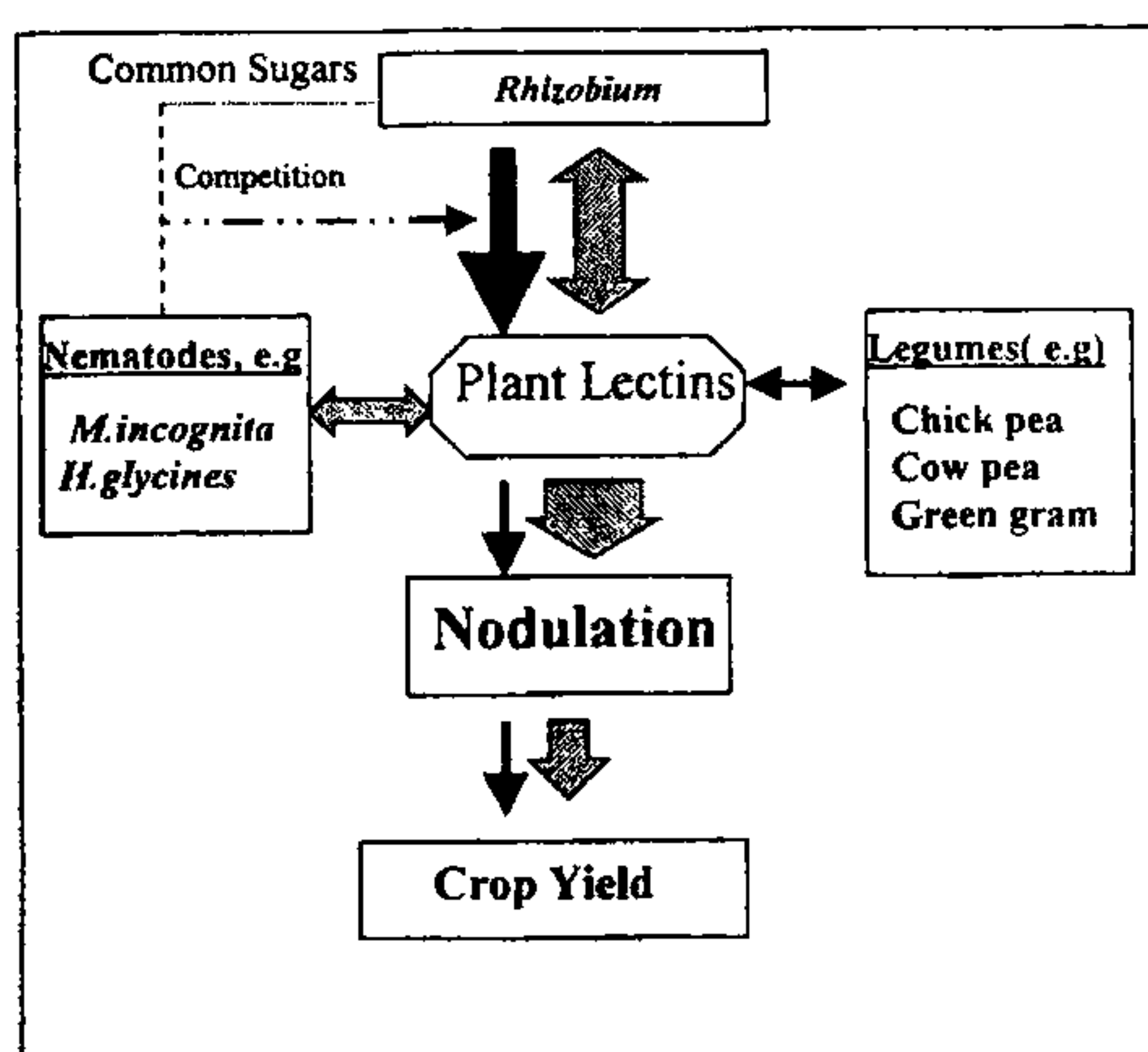

Figure 1. Competitive inhibition of Rhizobium–legume interactions by root-knot nematodes and its possible effects on crop yield. (Dark arrow depicts inhibition/reduction in nodulation and yield).
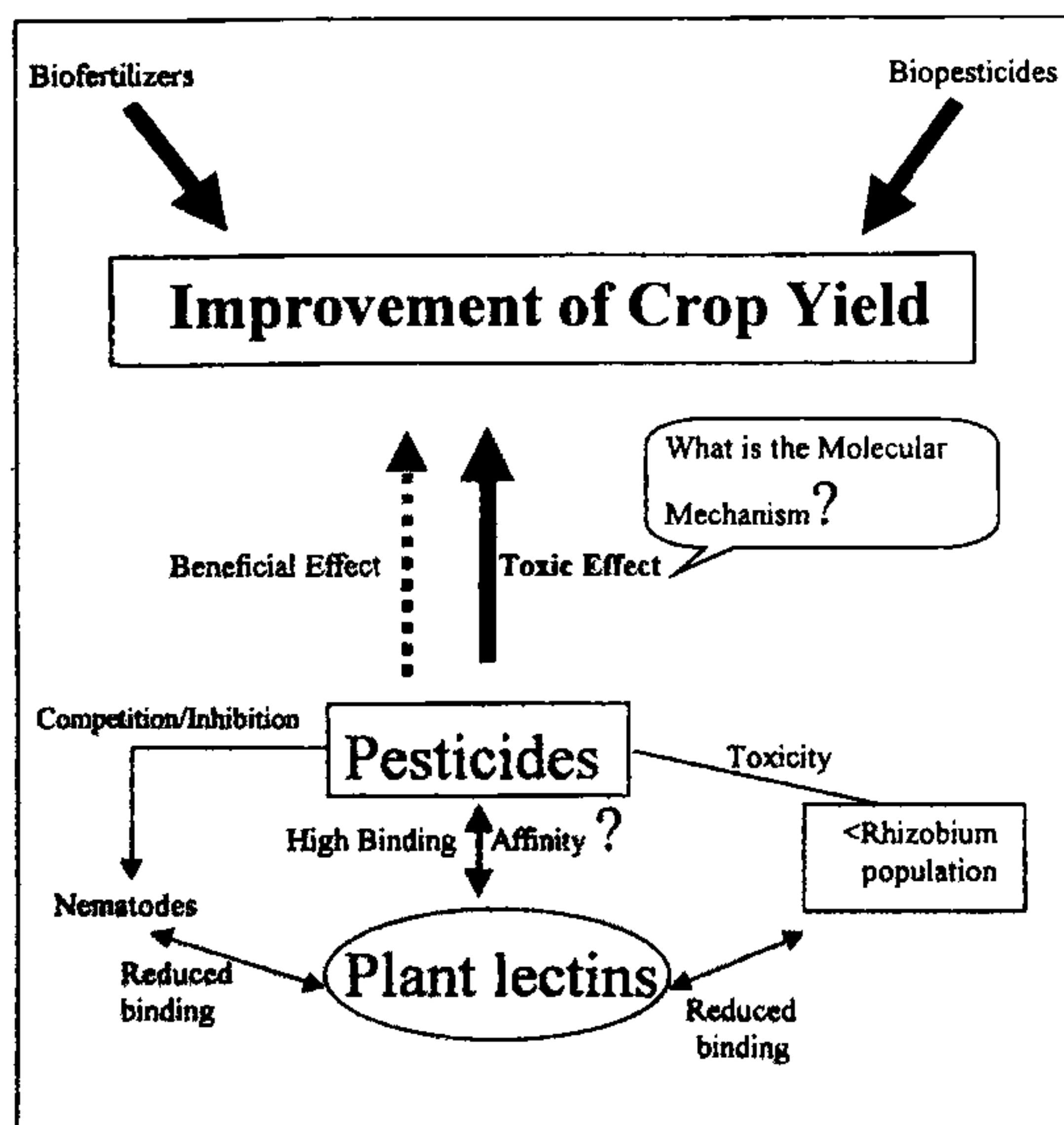

Figure 2. Antagonistic effect of agrichemicals on lectin mediated Rhizobium–legume interactions. The model shows interrelationship between the root-knot nematodes, Rhizobium and plant lectins.

Agrichemical–lectin interactions – A model study

In the process of Rhizobium–legume symbiosis, the microbiologists, in general, have given more attention to the bacterial partner, while plant physiologists, biochemists and agronomists have emphasized more on the host. In fact, a complete study of the effects of pesticides should involve both the Rhizobium and the legumes. While a considerable number of plant lectins have been isolated and characterized with respect to their structural and functional specificity and carbohydrate binding properties[31–35], precise information on the nature of their interactions with agrichemicals and soil microflora and the low and high affinity-binding sites on lectins is scarce in the literature. Paucity of data on this particular aspect, prompted us to emphasize the role of lectins in Rhizobium–legume symbiosis, their biochemical characterization and interactions with agrichemicals. Our recent studies based on fluorescence spectroscopy and circular dichroism (CD) could serve as a model for understanding the agrichemical-lectin interactions. In this study a bipyridinium herbicide, paraquat (PQ) has been chosen as a representative agrichemical and its in vitro interaction with the affinity purified Canavalis ensiformis (Con A) lectin was studied. The interaction was assessed by quantitative determina-
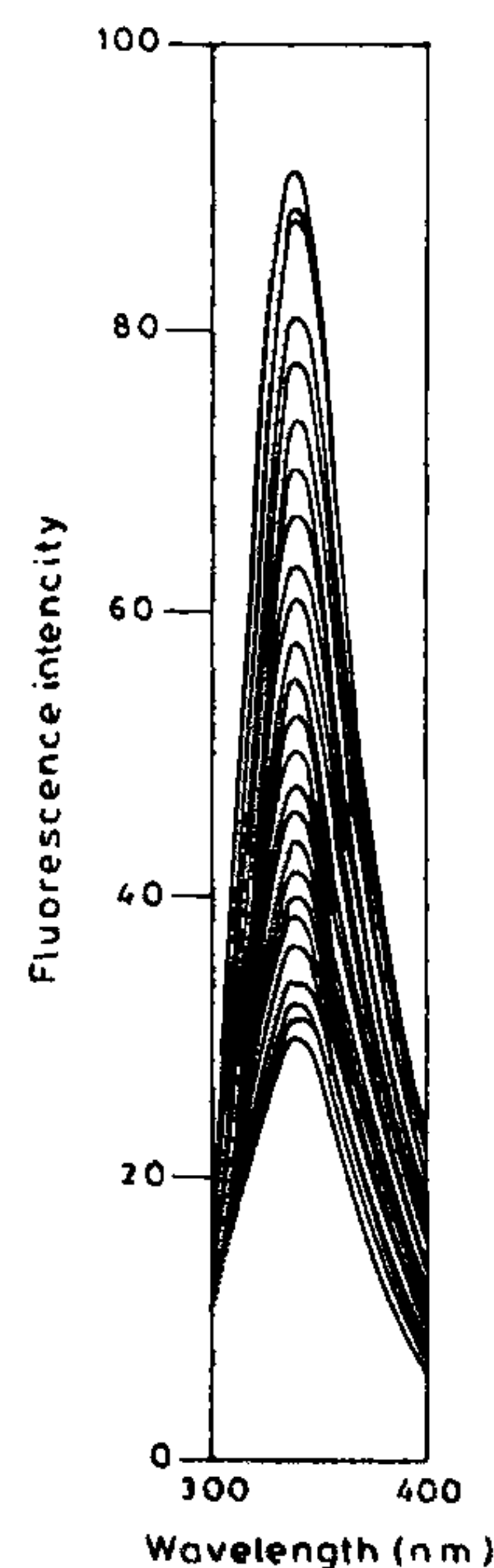

Figure 3. Emission spectra of Con A lectin, in the absence (uppermost curve) and presence of increasing amounts of paraquat, obtained in 0.05 M Tris–HCl, pH 8.0, I = 0.15 at 28°C on Shimadzu Spectrofluorometer RF-540 equipped with data recorder DR-3. The molar ratio of paraquat to Con A were (top to bottom) 0.0, 0.5, 1.0, 23. The excitation wavelength and slit width were 280 nm and 5 nm, respectively.
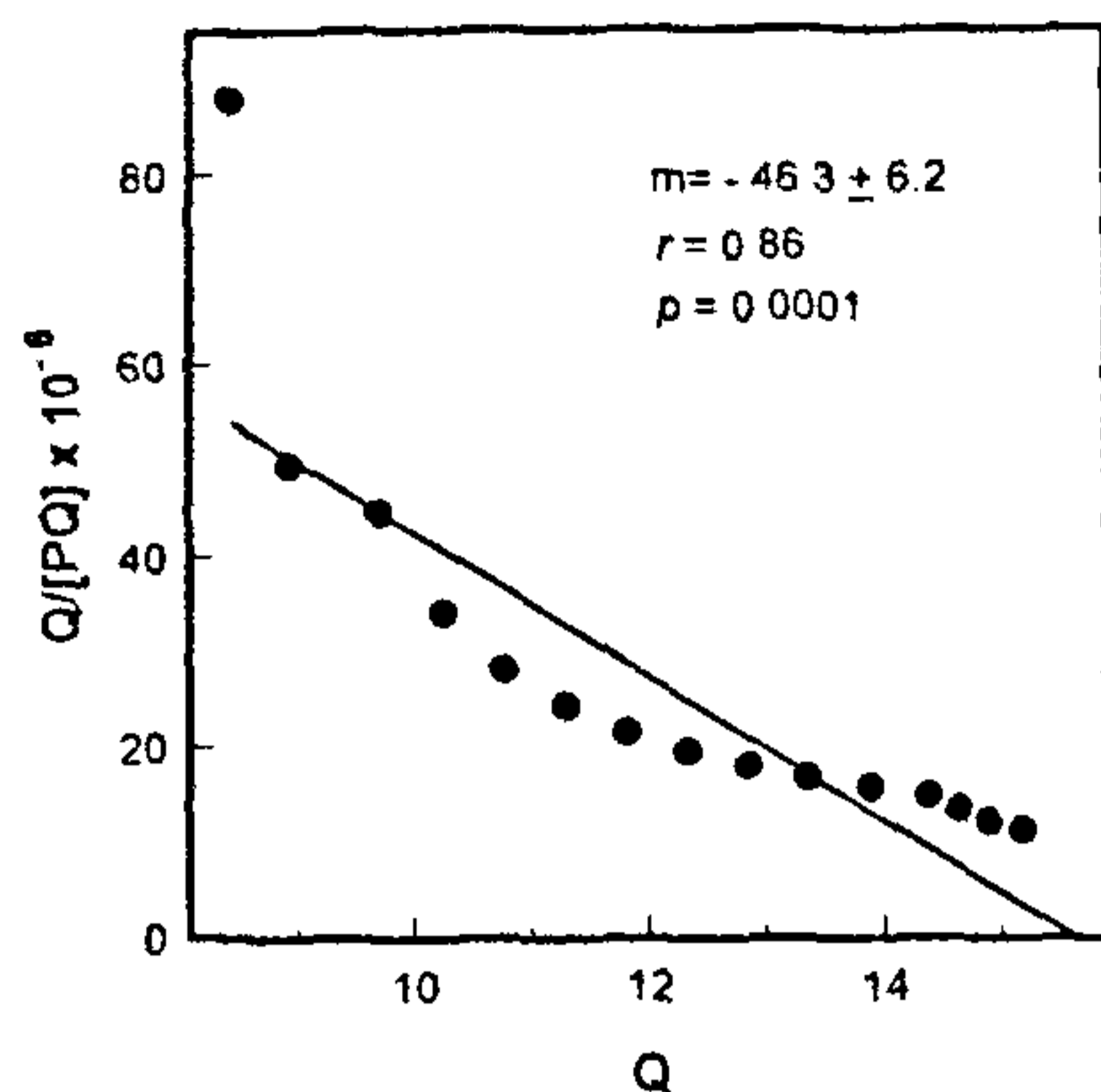
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Figure 4. The Scatchard plot for the binding of paraquat to Con A lectin. The values for Q/[PQ] and Q were obtained using the mathematical derivations described in the text and plotted as specified[36].
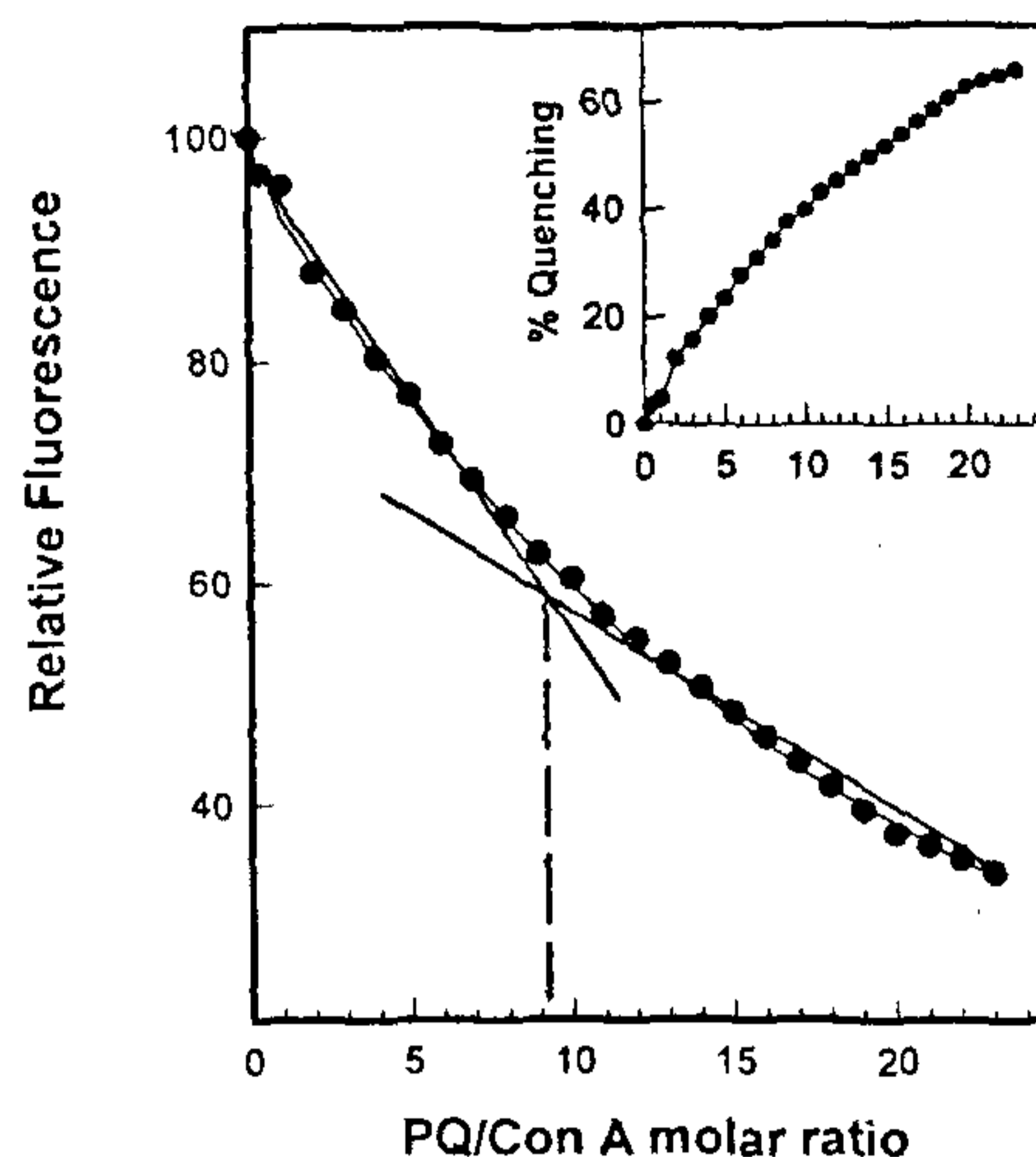

Figure 5. Binding isotherm of paraquat with Con A lectin at varying PQ/Con A molar ratios. The relative fluorescence was obtained from the spectra shown in Figure 3. The inset shows the % quenching as a function of PQ/Con A molar ratio.

tion of changes in the intrinsic fluorescence of Con A lectin at varying concentrations of PQ. The emission spectra at different paraquat/lectin molar ratios in the wavelength range of 300–400 nm are shown in Figure 3. The data clearly show PQ concentration-dependent reduction in the fluorescence intensity, suggesting significant interactions between PQ and lectin. The relative fluorescence was determined at an emission maximum of 340 nm, considering the intrinsic fluorescence of the PQ free lectin as 100. A least square analysis of the initial linear points on the plot of relative fluorescence versus PQ/lectin molar ratio yielded the maximum quench (m), following the relationship $F = Fo - mR$ (ref. 36). The fluorescence quench titration curve shown in Figure 4 revealed significantly high binding of PQ with Con A lectin. The binding isotherm shown in Figure 5 exhibited at least 25 PQ binding sites on the Con A lectin. Amongst these, nine were found to be high affinity sites and the remainder were loose sites. The Scatchard analysis in Figure 4 demonstrated the binding constant and capacity of Con A lectin to be 4.7×10^6 l/mole and 16.7, respectively. Similarly, the CD data also exhibited the herbicide concentration-dependent changes in the CD spectral intensities in the region of 275–285 nm and 320 nm, indicative of the conformational changes in Con A tertiary structure (our unpublished observation). The near-UV CD has been very commonly used for studying the molecular interactions including those between protein and ligands[37–39]. Aromatic side chains are frequently found at the ligand binding sites and have been utilized as sensitive probes of protein conformation and ligand binding[40,41]. Chemical modification studies revealed that most of the near UV CD intensity is related to tryptophan[42]. The near-UV CD spectra of paraquat-treated Con A also exhibited a negative band at 276 nm attributable to the tryptophan residue. Similar to Con A, the legume lectins also exist as oligomers, the nature of which has important functional implications[43,44].

CD data on Con A–PQ interactions indicated a monomeric form with increasing herbicide concentration. The lectin in a monomeric state, although has reduced tertiary structure, retains its ligand-binding activity to a considerable extent[45].

In conclusion, the high binding affinity of the test herbicide to lectin clearly supports the hypothesis of reduced nitrogen fixation due to competition of pesticides for the rhizobial-binding sites (lectins) on the surface of the legume root hairs. We need to initiate studies afresh on the mechanism of interactions of plant lectins with the commonly used agrichemicals. This will provide vital information regarding their role as suppressors of the lectin–rhizobial binding which, consequently, retards the Rhizobium–legume symbiosis. A database, if generated for the binding affinities of pure lectins with a series of commonly used agrichemicals could supplement the existing criterion for pesticide regulations. This will spur the government agencies in stringently enforcing the recommendations for the optimum usage of the selected agrichemicals. It is also urged upon the scientific community to develop ecofriendly and cost-effective alternatives for specifically improving the legume crop productivity and agricultural sustainability, in general.

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