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Effect of coal-based acid on bioactivity of the herbicide

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ABSTRACT

Water-soluble coal-based acids (WSCA) obtained by oxidation of Wuchuan weathered lignite (W-WSCA) or by extraction from the Jincheng weathered coal (J-WSCA), enhanced the biological activity of tribenuron-methyl, an herbicide. In the presence of the WSCA, the ED_{90} values for tribenuron-methyl were increased by a factor of 4.28 (W-WSCA) or 2.52 (J-WSCA). FTIR analysis indicated that the main interaction between WSCA and herbicide was via hydrogen bonds. NMR proved the results of FTIR. The established molecular dynamics simulation indicated that the more open structure favored the interaction between the herbicide and additive. The strong interaction between herbicide and additive and consequent marked change in herbicide structure are probably related to the improved biological activity of the herbicide.

1. Introduction

Pesticides, fungicides and herbicides play an important role in agriculture. It has been reported that losses due to insects, plant diseases and weeds consume 10%~15% of the total agricultural product but pesticides, fungicides and herbicides can decrease 15%~30% of the loss. However, the application of the agricultural chemicals brought about many serious side effects such as environmental pollution and harmfulness to human and animals. To reduce the harmful effects of agricultural chemicals, the strict environmental regulations have been introduced so that the process of developing a new agricultural chemical and the confirmation of its environmental suitability has become very expensive, which can take decades. A more effective approach is to search for harmless natural adjuvant which will increase the biological activity of agricultural chemicals.

Many studies have shown that the pesticides and herbicides in soil and water may interact with humic substances (HSs), the end products of chemical and biological degradation of plant residues [1,2]. HSs are the most widespread natural non-living organic materials, occurring in all terrestrial and aquatic environments and affect degradation and detoxication of the pesticides, residue persistence and monitoring, mobilization and transport, bioavailability and phytotoxicity, and bioaccumulation [3,4,5]. In general, the modes of interaction include adsorption, partitioning and solubilization, catalysis and dealkylation, and photosensitization [6]. HSs are well recognized and widely applied as a fertilizer and plant growing regulator. Patents have claimed the use of humic acid as an adjuvant for slow release of pesticide fertilizer compositions [7,8]. Substances similar to soil-derived humic acid can be obtained from coal and will be referred to as water-soluble coal-based acids (WSCA). Two kinds of WSCA can be distinguished (1) obtained by simple extraction procedures (2) formed by oxidation-degradation of coal. Our patents showed that, like soil-derived humic acids, WSCA enhanced the bioactivity of fungicides and insecticides [9,10]. So far little is known about the mechanism that humic acid affects the biological activity of agricultural chemicals.

In this study the adjuvant effect of two kinds of WSCA on a herbicide, Tribenuron-methyl (T), is studied to supplement the earlier work on insecticides and fungicides [9,10]. T was chosen because it is one of the most effective herbicides

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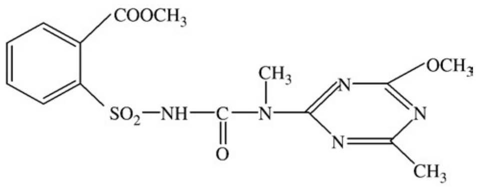


Fig. 1 – Chemical structure of tribenuron-methyl (T).

known against dicotyledon weeds. The continuous use of T quickly leads to the tolerance of herbicide, thus WSCA as a surface adjuvant, which improves the activity, reduces the amount required and slows down the buildup of resistance, was proposed. The interaction between WSCA and T was elucidated by analysis of the structure of the separate components and their reaction product using FTIR, ^{1}H NMR and computer aided molecular design (CAMD).

2. Experimental

2.1. Materials

2.1.1. Preparation of WSCA

To prepare W-WSCA, Wuchuan weathered lignite was first oxidized and degraded by heating it with HNO_3 for 2 h using Zn as catalyst at 80–100 °C. The ratio of solid to liquid in the mixture was then adjusted to 1:2 ~ 1:4 with water. The oxidized product was heated with NaOH for 2 h at 80 ~ 100 °C, and then concentrated H_2SO_4 was slowly added drop wise (about 2 ml/500 ml mixtures) with stirring. The pH value was monitored during the addition and subsequently adjusted to 4–6 with HNO_3 if necessary. The liquid phase was separated from the solid by centrifugation and the liquid dried under an infrared lamp at 70 °C to give W-WSCA. H-WSCA was prepared from Huolinhe lignite by the same procedure. To prepare J-WSCA, Jincheng weathered coal was mixed with water at a water/coal ratio of 10:1 with stirring. The solid residue was separated by centrifugation, water was added to it to give a water/residue ratio of 10:1, the pH value was adjusted to 1.2–1.5 with H_2SO_4, and the mixture extracted for 1 ~ 2 h in a water bath at 60 ~ 70 °C. The liquid was separated by centrifugation and dried under an infrared lamp at 70 °C. The product was purified by extraction in acetone and then dried to give J-WSCA after acetone removal.

The total amount of acidic oxygen functional groups of WSCA was determined with the method by Li [11]. About 50 mg of each sample was accurately weighed and added to 10 ml of 0.1 mol/L NaOH plus 25 ml $BaCl_2$. After stirring and separation by centrifugation, excess NaOH of 25 ml supernatant was then titrated using 0.1 mol/L HCl by a “Metrohm 736 GP Titrino” automatic titrator. The end point was used for calculation.

Carboxylic functional group was analyzed with the method by Li [11]. About 50 mg of each sample was accurately weighed and added to the centrifugal tube with 10 ml of 0.1 mol/L NaOH plus the equal HCl in order to separate HA out. Then 10 ml of 0.5 M/L $Ca(CH_3COO)_2$ was added to the tube with stirring and after centrifugation 25 ml supernatant was then titrated with 0.1 M/L NaOH using a “Metrohm 736 GP Titrino” automatic titrator. The end point was used for calculation. The amount of phenolic hydroxyl functional group was the difference between acidic oxygen functional groups and carboxylic one.

Coagulation value was tested following the method by Li [11]. 2.5 ml of different concentration $CaCl_2$ was added to a series of cuvette with 0.02% WSCAs and stirred, then to stand overnight. They were checked the next day to see if the mixtures remained homogeneous or if coagulation had occurred, and the concentration of $CaCl_2$, which led to coagulation, was confirmed. The test was repeated in different concentration range of $CaCl_2$ in order to give the accurate coagulation value.

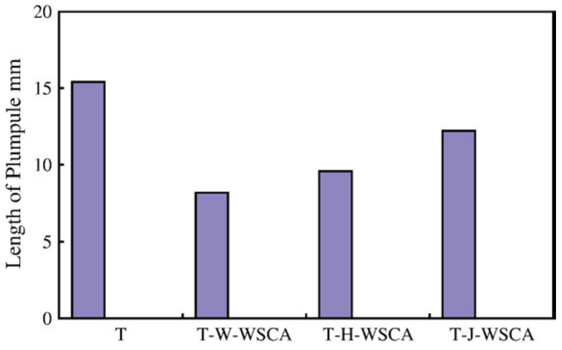


Fig. 2 – Effect of WSCA on the restraint of T on maize plumule.

2.1.2. Tribenuron-methyl

Tribenuron-methyl (T) for analytic measuring (99.99% active ingredient) and for bioassay (75% active ingredient) was supported by E. I. du Pont de Nemours and Company. The chemical structure was shown in Fig. 1.

2.2. Instrumentation

An EA1108 elemental analyzer (Carlo Erba) was used for determining elemental composition. The surface tension was

Table 1 – Proximate analysis of W-WSCA and J-WSCA

Samples	Elemental analysis/W% daf					Functional Groups (mmol/g$_{daf}$)		M_{ad} %	A_d %	HA_{daf} %	pH	Coag. value mmol/L
	C	H	O	N	S	OH_{ph}	COOH					
W-WSCA	60.15	3.65	28.52	2.38	3.38	2.96	3.46	8.71	33.33	58.12	4.0	36
H-WSCA	60.91	3.62	29.87	3.04	0.59	1.92	4.25	9.56	41.26	39.25	3.5	36
J-WSCA	50.01	3.14	44.57	0.91	0.87	1.52	8.96	13.2	5.50	94.50	1.0	0.6

Table 2 – Dose–response assays of T, T–W-WSCA and T–J-WSCA

Herbicide	Linear regression $y=a+bx$	ED_{50} g/ha	r	Index of relative toxicity
T	$y=5.61+0.56x$	1.2	0.9958	100
T–W-WSCA	$y=5.88+0.72x$	0.9	0.9942	133.3
T–J-WSCA	$y=5.77+0.65x$	1.05	0.9975	115.4

Table 3 – Index of relative toxicity of herbicide at different ED_{values}

ED_{values}	Index of relative toxicity		
	T	T–W-WSCA	T–J-WSCA
ED_{30}	100	100	100
ED_{50}	100	133.3	115.4
ED_{70}	100	212.2	161.9
ED_{90}	100	428.0	252.0

measured with a Processor Tension Meter (KRüSS). The contact angle of HSs on the surface of plant leaf (*Amaranthus retroflexus* L.) was measured by Face Contact Angle Meter (KYOWA KAIMENKAGAKU). FTIR spectra (400–4000 cm^{-1}) were recorded in KBr pellets using a Unison SP1000. ^{1}H-NMR spectra were measured on a DRX300 instrument.

2.3. *Methods*

2.3.1. *Bioassay of tribenuron-methyl herbicide action*

Two bioassays were used. Firstly, maize plumules were used to compare the effects of W-WSCA, H-WSCA and J-WSCA on the bioactivity of T. Maize seeds were sown in agriperlite, watered and allowed to germinate in the dark at 26 °C for 2 days. The germinated seeds were placed on the surface of a layer of sterilized sand 3 cm thick in a glass beaker and covered with another thickness of sand. Then the beakers were saturated with 1 μg/ml of T without or with 5 μg/ml of W-WSCA, H-WSCA and J-WSCA. Each treatment was repeated for three beakers. The weeds were grown in a greenhouse with a photoperiod of 16 h (22–25 °C in the light, 16–18 °C in the dark). After a week, the length of the plumules was measured. A second bioassay compared the effects of W-WSCA and J-WSCA in pot trails *Brassia campestri* was planted with pot culture in a greenhouse in order to measure the dose–response relation and obtain an index of relative toxicity. When the weed had developed one leaf, herbicides were applied with spraying. T concentrations of 0, 0.30, 0.90, 2.70, 8.40, 24.30, 72.90 g a.i./ha (active ingredient of T in a hectare) were used. The concentration of W-WSCA or J-WSCA was 10 g/ha. The fresh weight of plant above the soil surface was measured 14 days after the herbicide had been applied. Three replicates were tested for each set of conditions. The ratio of fresh weight without herbicide to fresh weight with a particular dose of herbicide was taken as the measure of relative activity of T, T–W-WSCA and T–J-WSCA.

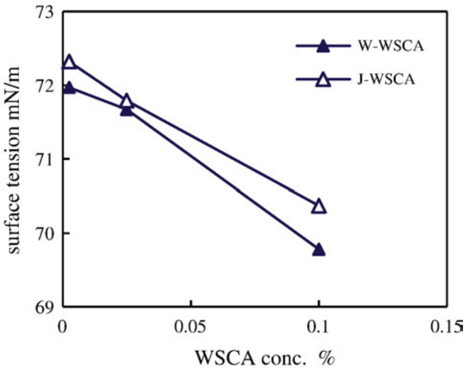


Fig. 3 – Effect of WSCA on surface tension.

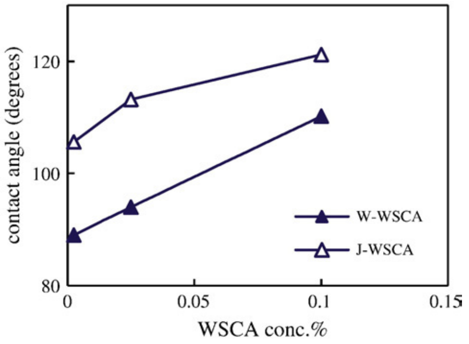


Fig. 4 – Effect of WSCA on contact angle.

2.3.2. *Field trials of T herbicide action*

The field trials of T, T–W-WSCA and T–J-WSCA herbicide action were carried out in a wheat field in Taiyuan, Shanxi province starting on Apr. 23, 2000. The concentration of T was 20 g/ha in all tests. The concentrations of WSCA were 18.7 and 187 g/ha, and the test area was 30 m^2 with 4 replication located

Table 4 – Adjuvant effect of WSCA on T against weed in wheat field

Herbicide	Effectiveness at 20th day after herbicide applied					
	Aretisia	*Chenopoaium serotinium*	*Bothirospeimum chinense* bge	*Lepiaium*	*Amaranthus retroflexus* L.	*Convolvulus arvensis*
T	69.7 b B	54.7 a AB	68.4 b B	44.9 b B	43.0 b B	73.9 c B
T–W-$WSCA_1$	86.4 a A	71.7 a A	82.0 a A	88.9 a A	83.3 a b A	92.2 ab A
T–W-$WSCA_2$	94.6 a A	87.9 a A	85.7 a A	95.8 a A	92.7 a A	96.9 a A
T–J-$WSCA_1$	88.2 a A	68.8 a A	80.8 a A	91.8 a A	87.9 a A	89.5 b A
T–J-$WSCA_2$	95.4 a A	80.6 a A	88.9 a A	95.3 a A	95.3 a A	98.4 a A

$WSCA_1$ — concentration was 18.7 g/ha $WSCA_2$ — concentration was 187 g/ha.

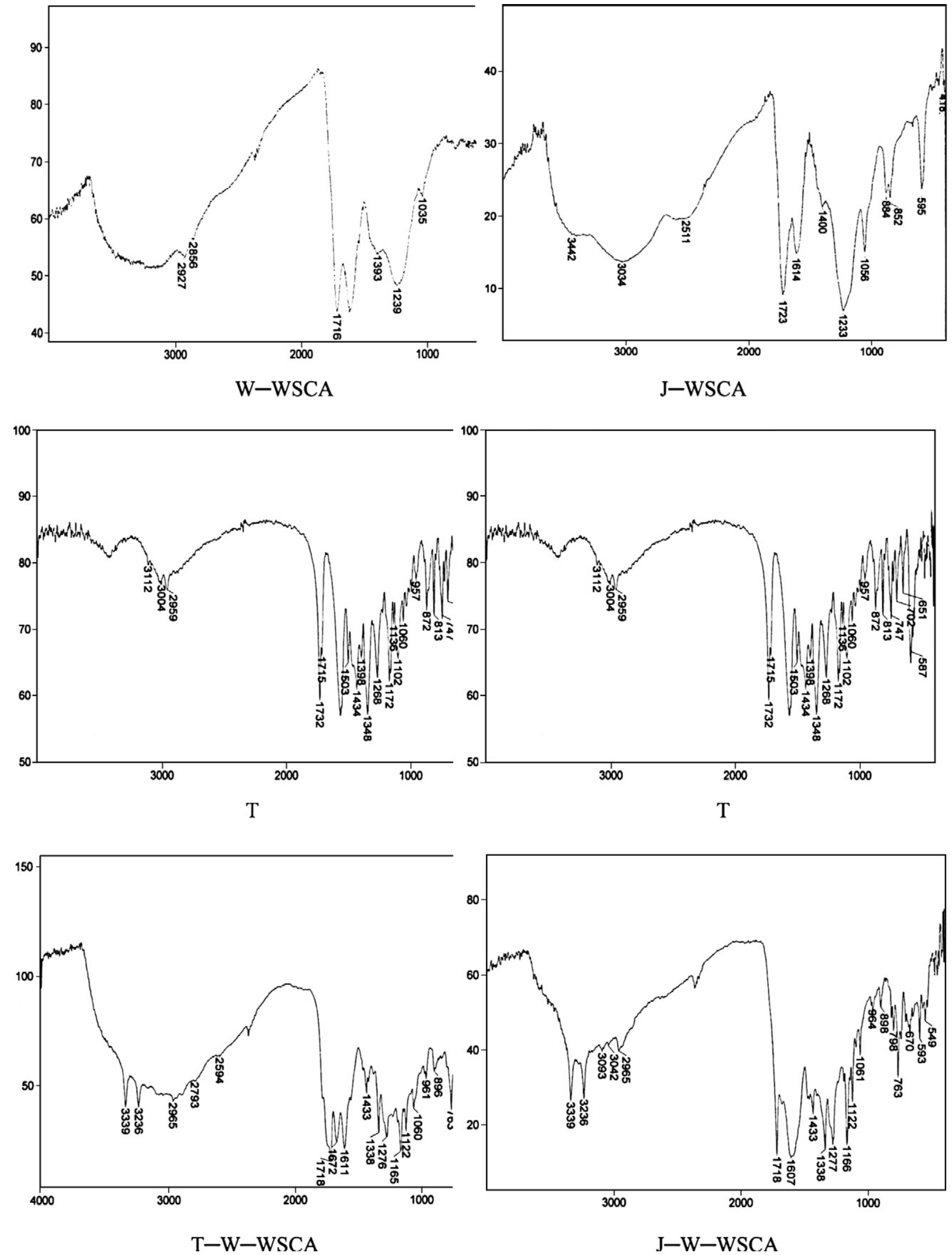
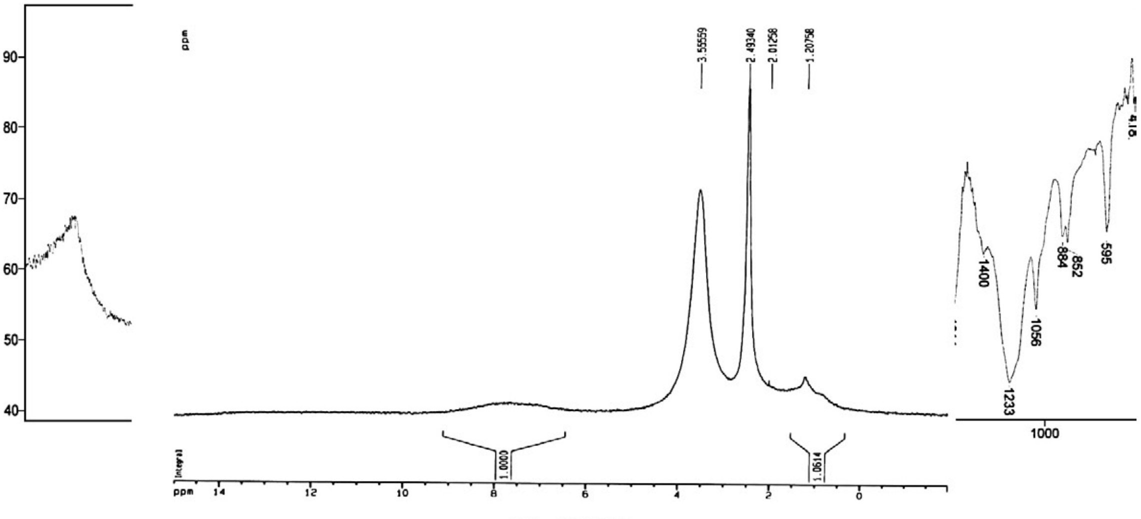
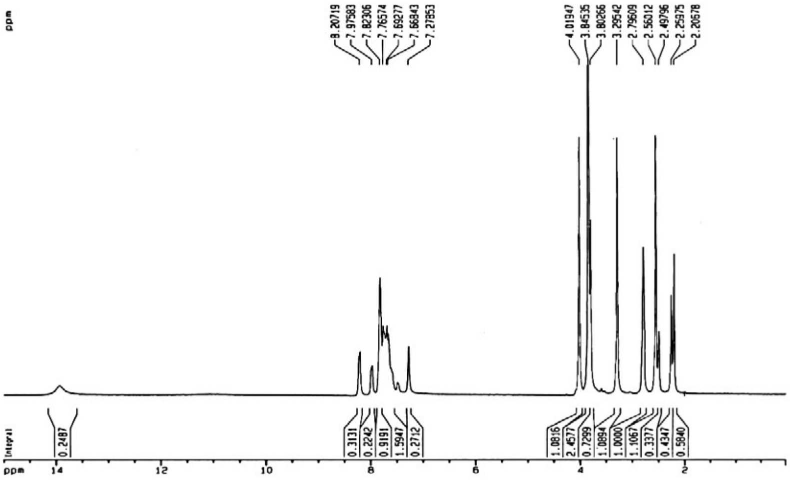


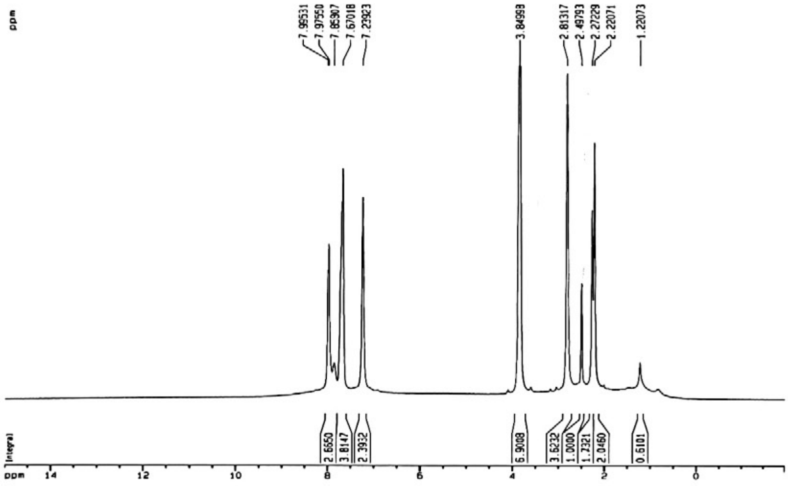
Fig. 5 – FTIR spectra.



W—WSCA



T



T—W—WSCA

Fig. 6 – ^{1}H-NMR spectra.

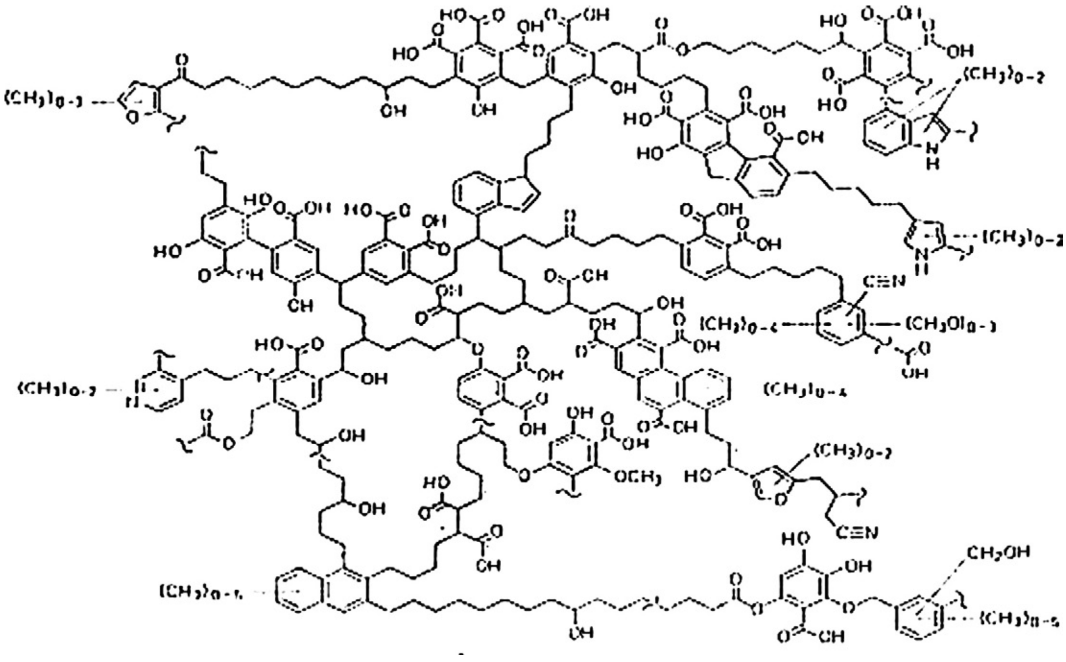


Fig. 7 – Chemical structure of humic acid built by H-R. Schulten [13,14].

in a random arrangement. The fresh weight of weed was measured on the 21st day. Duncan's test was used for statistical analysis.

Computer-aided Molecular Design (CAMD) was carried out on a PC using the programme HyperChem.

3. Results and discussions

3.1. *Characteristics of WSCA*

The elemental analysis and properties of WSCA are listed in Table 1. The functional groups are of vital importance to the characteristics of WSCA. The amount of phenolic hydroxyl group was highest in W-WSCA, while that of carboxyl group and HA in J-WSCA. Adding large amounts of Calsulmag or other calcium sources to WSCA caused coagulation and clumping, making it impractical for WSCA application. The higher the coagulation value is, the stronger the capacity of inhibiting coagulation is. WSCA form highly stable complexes with Fe^{3+}, $Ca^{2+}$and Mg^{2+}, inhibiting coagulation in solutions containing these ions. Coagulation value means the ability of WSCA against electrolyte. The coagulation value of WSCA obtained by oxidized degradation (W-and H-WSCA) was about 60 times as large as that existing in coal (J-WSCA). It showed that the molecular weight of W-WSCA or H-WSCA was lower than that of J-WSCA with different distribution in functional groups.

3.2. *Result of bioassay*

The first bioassay, maize plumules were used for qualitatively comparing the adjuvant effect of WSCA obtained by simple extraction procedures (J-WSCA) and formed by oxidation-degradation of coal (W-WSCA, H-WSCA) on bioactivity of T.

Fig. 8 – Molecular modeling of T optimized.

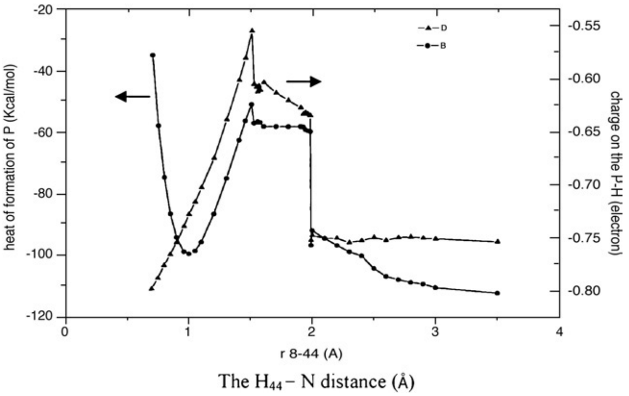


Fig. 9 – Changes of heat of formation and charge of T with distance of N.H.

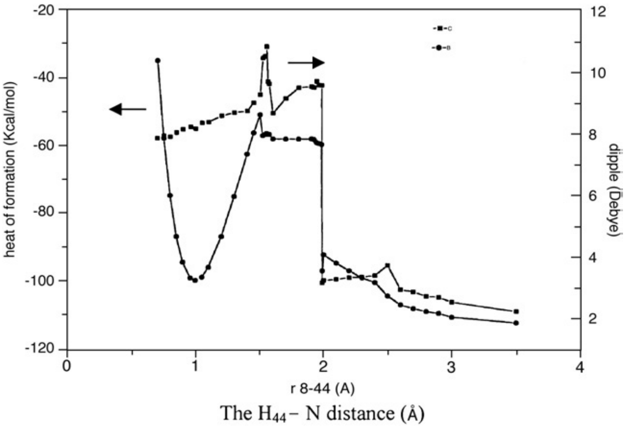


Fig. 10 – Changes of heat of formation and dipole of T with distance of N.H.

The shorter the length of plumule was, the stronger the bioactivity of herbicide was. The result as shown in Fig. 2 pointed out that addition of WSCA to T reduced the average plumules length in all cases, so that all WSCA enhanced the bioactivity of T. Moreover, WSCA formed by oxidation-degradation of coal (W-WSCA, H-WSCA) was more effective than that obtained by simple extraction (J-WSCA) and W-WSCA was the most effective.

The second bioassay is to obtain index of relative toxicity for the quantitative adjuvant effect of WSCA. Table 2 listed the parameter of dose–response assays of T, T–W-WSCA and T–J-WSCA. ED_{50} means the concentration of herbicide when the death ratio of weed is 50%. Index of relative toxicity is in ratio of ED_{50} of single agricultural chemical to ED_{50} of the agricultural chemical added adjuvant. Table 3 shows that the index of relative toxicity increased with increasing effective dose of T. According to the IUPAC standard, an adjuvant is defined as significantly effective if the index of relative toxicity is over 120 [12], so that W-WSCA was significantly effective at ED_{50} and both WSCAs were effective at ED_{70} and ED_{90}.

The field trials of T, T–W-WSCA and T–J-WSCA against weeds in the wheat field also showed a significant adjuvant effect of WSCA on T. The results are listed in Table 4. The effectiveness differed with different weeds. For *Lepiaium* and *A. retroflexus* L, the effectiveness of T with WSCAs was 2 times than that of T without WSCA. For the other weeds, WSCAs raised T effectiveness by 20% in all cases. Furthermore, the effect of T–WSCA with a higher concentration of WSCA was greater than that at the lower concentration of WSCA. The statistic tests, for which same letter means the same level of adjuvant, showed that WSCA significantly enhanced the biological activity of T again all the weeds, but the difference between W-WSCA and J-WSCA was not significant.

3.3. *Mechanism of adjuvant effect*

So far little is known about the mechanism that humic acid affects the biological activity of agricultural chemicals. A possible explanation of the adjuvant activity of the WSCAs is that they are surface-active agents and thus enhance the interaction between T and the plant. The surface tension and the contact angle of the solution of WSCA on the plant leaf were shown in Figs. 3 and 4, respectively. It is found that WSCA did improve the surface activity of the spray solution and the effect was larger at higher WSCA.

Chemical information concerning the interaction of WSCA with T was obtained using FTIR as shown in Fig. 5 and ^{1}H-NMR in Fig. 6. In WSCA, there was hydrogen bond by COOH (2800–2400 cm^{-1}) and by OH (3400 cm^{-1}). There was peak of 1239 cm^{-1} in W-WSCA or 1233 cm^{-1} in J-WSCA and the former was stronger than the later but there was no peak in 1100 cm^{-1}. It means OH formed hydrogen bond was from the phenolic hydroxyl group. After the interaction, the following changes were markedly. The hydrogen bond, N.H and O.H, were formed between WSCA and T due to the formation of two small sharp peaks in 3339 cm^{-1} and 3236 cm^{-1} and the peak of W-WSCA–T is slightly stronger than that of J-WSCA–T. The peak in 1233 cm^{-1}of WSCA disappeared and the intensity of

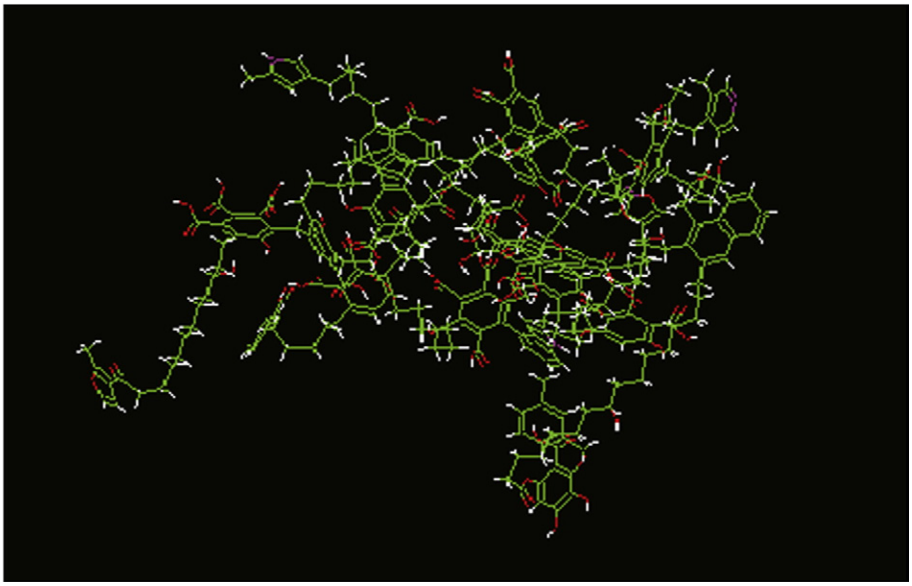


Fig. 11 – Optimized molecular model of WSCA.

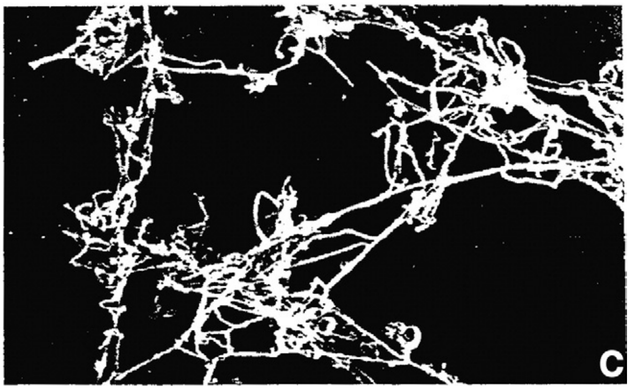


Fig. 12 – Transmission electron microphotograph of 0.01% humic acid solution.

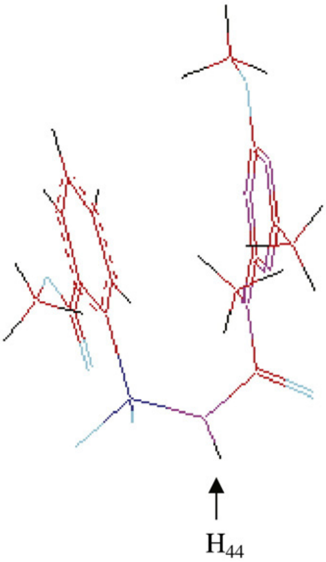


Fig. 14 – Molecular modelling of T after action with WSCA.

1718 cm^{-1} (C=O) and 1400 cm^{-1} (C.O) decreased. The specific peak of triazinyl of T disappeared. It is possible that H (H_{44}) in .H.NCO$_2$. of T formed hydrogen bound with hydroxyl O (O_{199}) in benzoic acid of WSCA. Furthermore, it was also proved by the ^{1}H-NMR spectra as shown in Fig. 6 because the peak at δ^{14} ppm of T disappeared after interaction. Thus, it is assumed that the hydrogen bond formed between phenolic hydroxyl group of WSCA and T enhanced the biological activity of T because the biological activity of W-WSCA was highest with the most phenolic hydroxyl group although the amount of humic acid in W-WSCA was lower than that of J-WSCA.

Molecular modelling is a new and helpful technology and used for agricultural chemicals. With the new approach it is necessary to build the molecular model of WSCA. Due to the complex molecular-chemical structure of humic substance, a number of chemicals and structures of humic substances have been proposed over the years. They consist of either polycyclic aromatics or aromatic moieties without consideration of significant contributions of aliphatic structures. The chemical structure concept of humic acid reported was some structural fractions and functional groups, leading to the difficulty to study the biological mechanism of humic acid in molecular level. Schulten present a structural concept shown in Fig. 7 for humic substance based on the extensive experiments [13,14]. The net structural model combined a lot of functional groups and structural units that were proved to be existed in humic acid such as polycyclic aromatics, aromatic moieties, alkylate and aliphatic structures etc. It had the elemental composition of $C_{308}H_{328}O_{90}N_5$ with a molecular mass of 5540.027 g mol^{-1} and an elemental analysis of 66.8% C, 6.0% H, 26.0% O, 1.3% N similarly to that of WSCA in this paper.

The optimized geometry of T was done by computer running based on the rule of minimum energy shown in Fig. 8 and the effect of the bond distance of H_{44}.N (R) on heat of formation, charge of molecule and dipole of T by HyperChem were present in Figs. 9 and 10. The results showed that the effect of *r* was very remarkable as shown in Fig. 8. The heat of

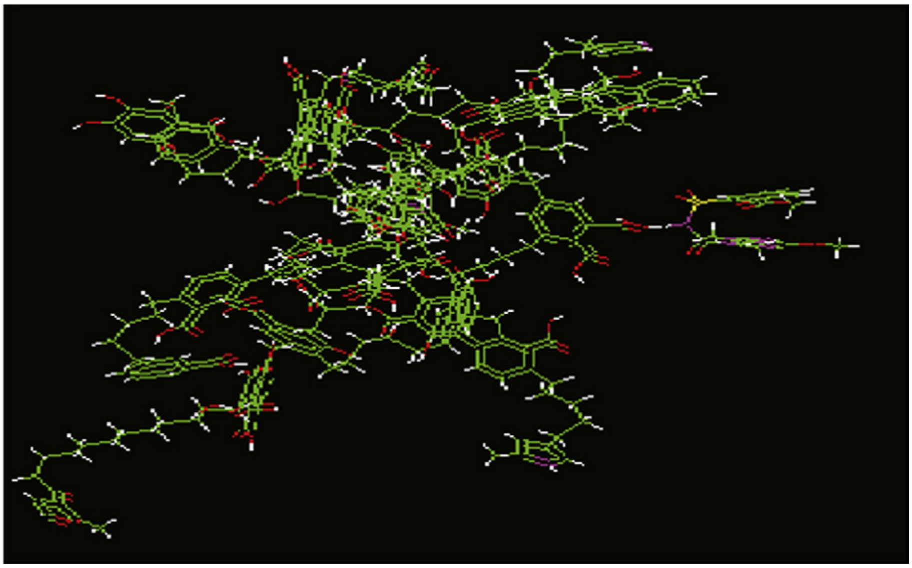


Fig. 13 – Optimized molecular model of T–WSCA.

formation went down sharply and began to rise up at 1.00Å, then accelerated up in 1.45 Å–1.57 Å and was further down with increasing *r*. The charge changed just against the heat of formation before 0.9 Å in Fig. 10, and then both changes in the same tendency. The dipole had little change between 0.7 Å—1.5 Å but sharply dropped in 2.0 Å. It means that the molecule of T transformed from ground state to activated state with increasing *r*, implying the dependence of T activity on the *r*.

The molecular model of WSCA based on the chemical structure of humic acid by Schnitzer was optimized by molecular mechanics and presented in Fig. 11. It can be found that the style of the molecular modeling of WSCA was similar to the result that M. Schnitzer observed with TEM in Fig. 11 [15], implying that the assumption used to construct the model is reasonable. FTIR and NMR data above suggested that H_{44} of N.H in T was with O_{199} of hydroxyl group in benzoic acid of WSCA in H-bond. The complex of T–WSCA was optimized by molecular mechanics again as shown in Fig. 12. The result indicated that WSCA changed the molecular structure of T to U style as shown in Fig. 13. It was reported that U style is the activated molecular structure of Sulfa agricultural chemicals [16]. The distance of O and H was 1.0404 Å.

4. Conclusions

WSCA can strongly enhance the biological activity of tribenuron-methyl again weed and the newly formed one was more effective than the originally existed one. FTIR and NMR analyses indicated that the main interaction between WSCA and herbicide was via hydrogen bonds. The molecular modelling of T after action with WSCA showed that WSCA changed the molecular structure of T to U style, which is the activated style (Fig. 14).

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