(12) UK Patent Application (19) GB (11) 2 269 165 (13) A

(43) Date of A Publication 02.02.1994

(21) Application No 9315734.5

(22) Date of Filing 29.07.1993

(30) Priority Data
(31) 92109075 (32) 30.07.1992 (33) CN

(71) Applicant(s)
Swallow Enterprises Co Ltd

(Incorporated in Hong Kong)

Rm 1503-4 Wing On Central Bldg,
26 Des Voeux Road, C, Hong Kong

(72) Inventor(s)
Fu Sheng Feng

(74) Agent and/or Address for Service
Page White & Farrer
54 Doughty Street, LONDON, WC1N 2LS,
United Kingdom

(51) INT CL[5]
A01N 33/12

(52) UK CL (Edition M)
C1B BAA

(56) Documents Cited
GB 2134505 A GB 2079605 A GB 2059412 A
GB 2052260 A EP 0220514 A EP 0190561 A
EP 0167776 A EP 0146017 A EP 0009696 A

(58) Field of Search
UK CL (Edition L) C1B
INT CL[5] A01N
ONLINE DATABASE: WPI

(54) An agricultural composition for promoting photosynthesis in plants

(57) This invention relates to an agricultural composition that may promote photosynthesis of plants, its method of preparation and application. The composition of the invention can accelerate the photosynthesis of different kinds of plants and thereby improve the quality of farm crops and their yields as well. The composition comprises choline or its derivatives or agriculturally acceptable salts and an agriculturally acceptable carrier.

GB 2 269 165 A

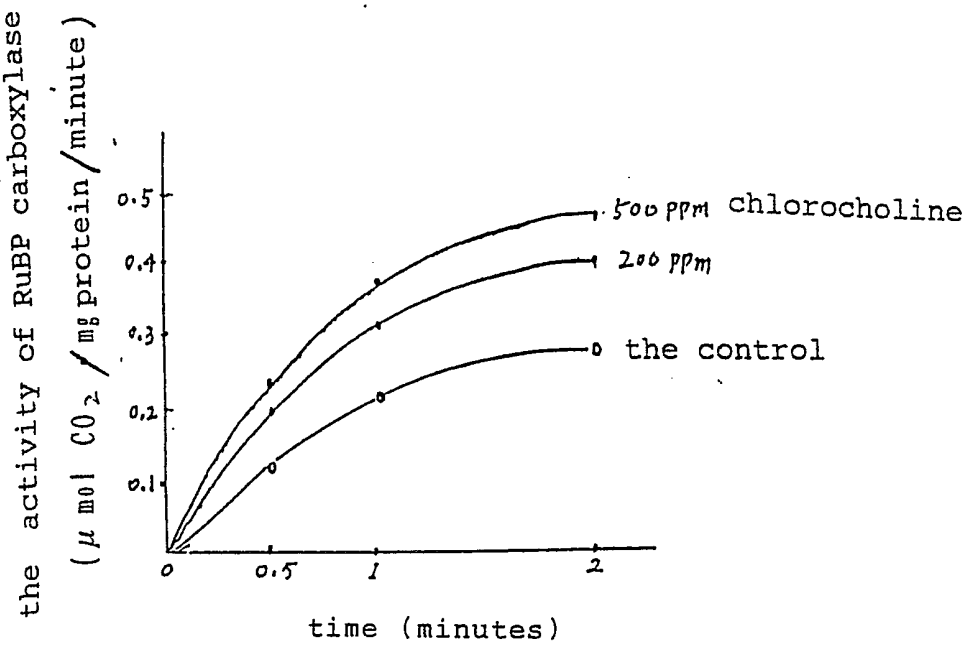

the activity of RuBP carboxylase
(μ mol CO_2 / mg protein / minute)
0.5
0.4
0.3
0.2
0.1
0
0.5
1
2
time (minutes)
500 ppm chlorocholine
200 ppm
the control

Fig. 1

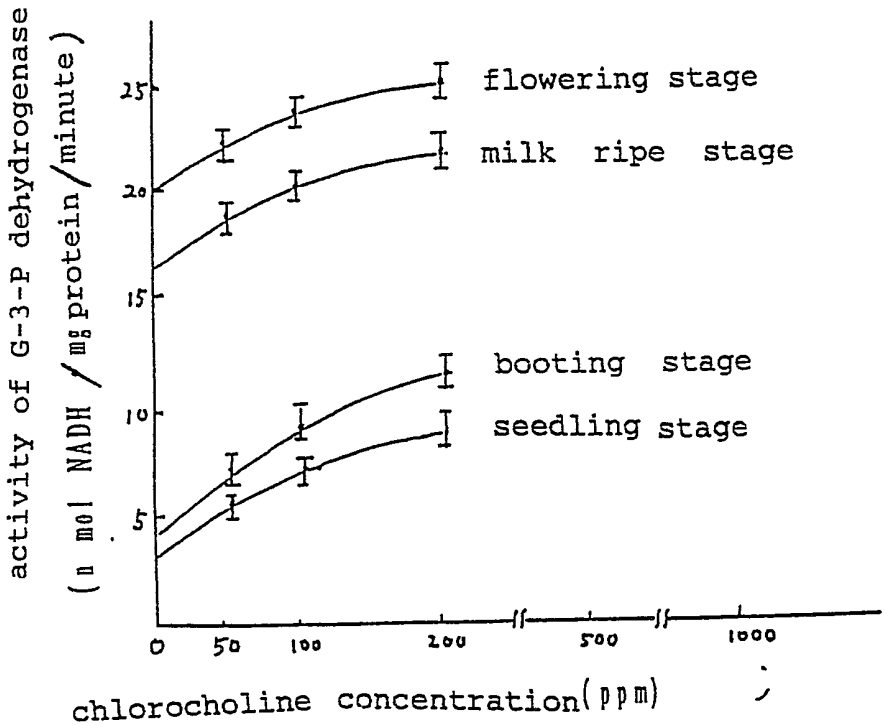

activity of G-3-P dehydrogenase
(n mol NADH / mg protein / minute)
25
20
15
10
5
flowering stage
milk ripe stage
booting stage
seedling stage
0
50
100
200
500
1000
chlorocholine concentration(ppm)

Fig 2.

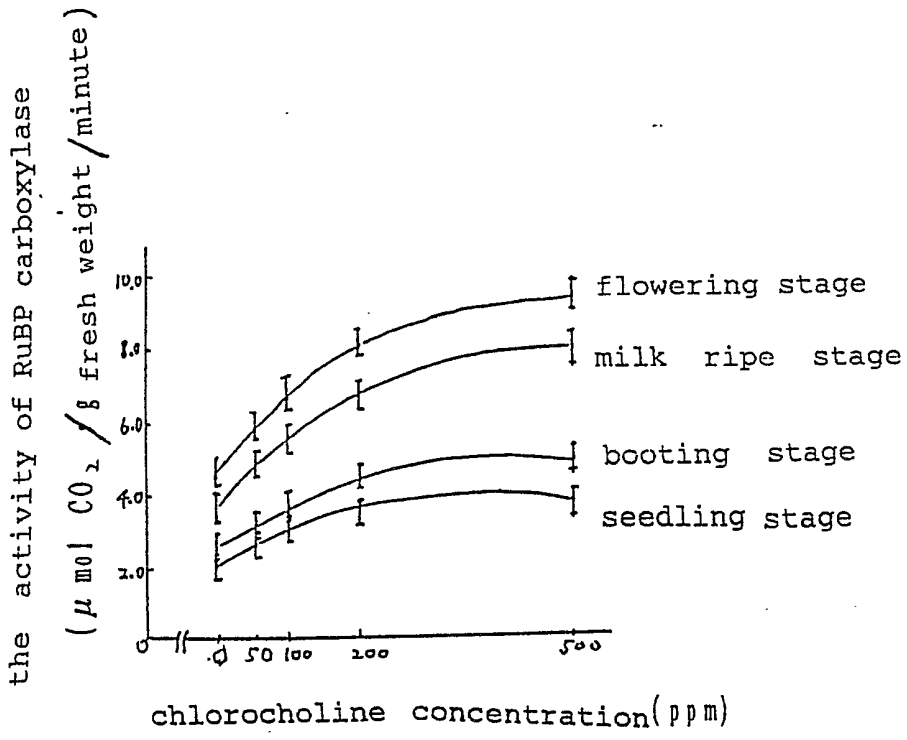

the activity of RuBP carboxylase
(μ mol CO2 / g fresh weight / minute)
10.0
8.0
6.0
4.0
2.0
0
50
100
200
500
flowering stage
milk ripe stage
booting stage
seedling stage
chlorocholine concentration(ppm)

Fig 3

Fig. 4

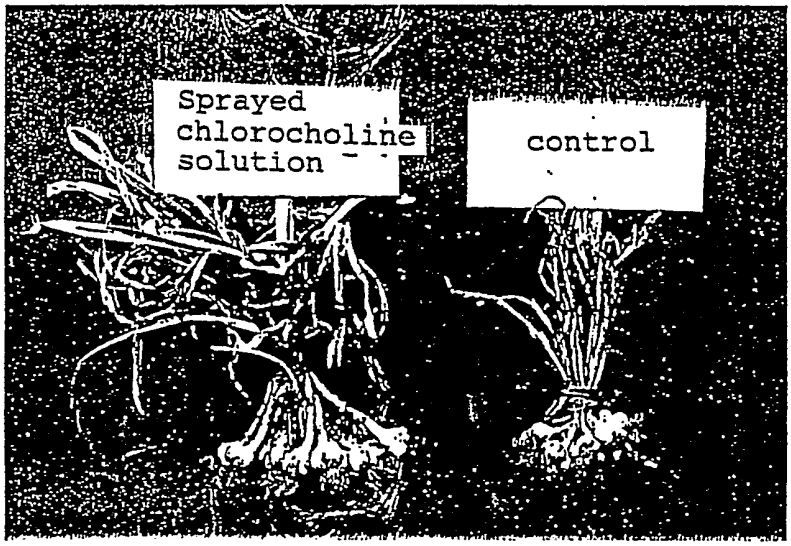


Fig. 5

A Sprayed chlorocholine solution

Fig. 6

B control

2269165

AN AGRICULTURAL COMPOSITION FOR PROMOTING PHOTOSYNTHESIS IN PLANTS

This invention relates to an agricultural composition that may promote photosynthesis of plants, its method of preparation and application. Particularly the invention involves the composition for promoting photosynthesis in plants, which mainly comprises choline or its derivatives or agriculturally applicable salts mineral elements, allantoin or its derivatives and an agriculturally acceptable carrier, the methods of preparation and application.

Chlorocholine is a feed additive for feeding animals, which has been widely used since 1940s. It promotes the utilization of methionine in animals. In plants, Farkas et al. (1985) found that the transformation speed of chlorocholine into phosphatidyl choline increased after infiltration of ^{14}C-mevalonate and sterol into kidney bean (Phaseolus vulgaris L.) leaves. Sfaudtlunder et al. (1982) found that the addition of chlorocholine into the culture of yeast induced the increase of phosphatidyl choline to a certain amount. Kates et al. (1975) reported that phosphatidyl choline

made up 27% of the total amount of phosphoipid in chloroplasts, 62% of that in the thylakoids and 24% of that in grana lamellae.

The rate of photosynthesis is related to the enzymatic level and activity of RuBP (ribulose biphosphate) carboxylase (Jensen, Ann, Rev. Plant Physiol.28:379-400,1972); as to the function of phosphatidyl choline, an important constituent of phospholipid to the enzymes, bound to membrane, some scholers considered it as an agent forming a necessary hydrophobic condition for the boundenzymes. Thus the activated enzymes and their substrate are under an optimum condition for enzymatic catalysis. (Tang, Zhangcheng. News-letters of Plant Physiology, 1983(3):8-14, 1981). Other scholars also indicated that phosphatidyl choline (70% linolenic acid) might renovate the activity of ATPase.

As we all know, the photosynthesis in plants may be divided into light reaction and dark reaction. ATPase is the key enzyme in light reaction and RuBP carboxylase and G-3-P dehydrogenase are the key enzymes in the dark reaction. The increase of ATPase activity may accelerate the formation of ATP which supplies energy for assimilation of CO . Increase in the activity of RuBP carboxylase will increase the amount of assimilating and fixing CO . The raise in the activity of G-3-P dehydrogenase will promote the content of photosynthetic product-sugar. The photosynthetic accelerator of this invention can raise the activities of the 3 key photosynthetic enzymes cited above.

Under illumination, plants carry on not only normal respiration, consuming energy, but also a kind of photorespiration which may consume about 40% nutrient acguired by the plants through photosynthesis. If photo respiration could be reduce to 50%, yields by plants above 10% might be increased. The photosynthetic accelarator of this invention being an ideal inhibitor for photorespiration, may reduce the photorespiration to about 50%.

At present the internationally used common photorespiration inhibitor is mainly sodium hydrogen sulphite, which can only check about 10-20% of photorespiration. However, the photosynthesis accelarator of the present invention may inhibit about 50% of photorespiration.

The invention for the first time indicates the use either of choline or its derivative or salts or of allantoin or its derivative to promote plant photosynthesis as well as to inhibit photorespiration. Until now, there are not yet any reports or inventions about the utilization of these compositions for photosynthesis of plants in experiments or application (subject indexing to world literature and appraisal in separate appendix), nor even the similar related reports.

At present with the explosion of world population and decrease of acreage per capita and the increasing demands on farm products all are extiemely urgent; especially in the Third World agricultural countries the situation of food shortage is more serious. The

photosynthetic accelerator of this invention may promote the absorption of light energy and the fixation of more CO to increase farm yield under the original cultivation condition. Therefore it is a effective measure to overcome food shortage and to increase the yield of economic crops to a large extent.

One aim of this invention is to provide an agricultural composition that may promote the photosynthesis in plants.

Another aim is to provide the method of preparation and application of the agricultural composition cited above.

The agricultural composition of this invention comprises mainly an effective amount of choline or its derivative or agriculturally acceptable salts and an agriculturally acceptable carrier.

This composition may also contain optionally an effective amount of mineral elements and/or allantoin or its derivatives.

The mineral elements contained in this invention function mainly in three aspects: (1) as a coenzyme component for photosynthetic key enzyme, (2) as an activator for photosynthetic key enzyme, (3) as a complex of photosynthetic product to accelerate the transportation in plant body.

The function of allantoin or its derivatives is to inhibit part of the reaction of photorespiration so that the organic substance

consumed in photo respiration is minimized and the accumulation of photasynthetic product maximized.

In the composition of the invention certain amount of auxiliaries, including pH value regulating substance (such as soda, sodium carbonate etc.), wetting agent and surfactant may be contained.

In the composition, the content of choline or its derivative or its agriculturally applicable salt is 0.001-99%, mineral elements 0-30%, allantoin or its derivative 0-99% and auxiliaries 0-25%.

An agriculturally applicable salt of choline or its derivative may be chloride, bromide, iodide, phosphate, hydrogen phosphate, dihydrogen phosphate, sulphate, hydrogen sulphate, carbonate, bicarbonate, hydrogen sulphite, tartrate and phosphatidyl compound of which chloride is preferred.

The mineral elements in the composition of the invention may be B, Mg, Zn, Mo, S, I, Na, Si, Mn, Cu, Fe, K, Ca, P, etc. or a mixture of two or more.

The agricultural composition of the invention may be used together with other pesticides, such as various kinds of organophosphorus pesticides (such as methamidophos, omethoate, monocrotophos etc.), pyrethrins (such as decamethrin etc.) and condensed amines etc. In so doing, the effects of both insect/fungi control and increase in yield can be obtained.

The composition of the invention may be used in any form, preferably in water solution.

The composition of this invention, as an accelerator of photosynthesis, may be applied in many ways; for example, it can be applied directly onto the leaves or seeds or indirectly to the medium where the plants or seeds grow. It may be applied in various forms, as spray, powder, emulsion or paste, in vapour or slowly releasing granules. It may be applied to any part of the plant body including leaf, stem, twig or root or even to the soil around the root, or the seeds before sowing. The composition herein may also be injected into the plant body or sprayed on vegetables by means of electric atomization or others in low volume. The form of preparation is dependant upon by the specific purpose of the design.

The preparations may be made in powder or granule form, in which the solid diluent or carrier may be kaolin, bentonite, white diatomite, dolomite, calcium carbonate, talc, magnisium oxide powder, bleaching earth, gypsum, diatomite, and pottery clay and the like filler. The granules can be ready made granules suitable for application into soil without the need for further treatment. These granules are made up of small globules of filler soaked in an active indigrent or with mixture of powdered filler and an active indigrent. The preparation for the seed dressing may include a kind of reagent (e.g. water) to adhere the preparation to the seeds. The preparation may also be in form of wettable powder or

in granules that can disperse in water; in these cases the preparation must contain wetting or dispersing agents to help them disperse in solution. These powder and granules may also contain filler and suspending agent.

The technical term "plant" used here implies seedling shrub or tree.

The technical word "agriculturally applicable" means "for agricultural and horticultural purposes".

The preparation method for the composition herein includes the mixing up of certain amount of choline or its derivatives or its agriculturally applicable salt with an agricultural carrier, then the optional adding of a certain amount of trace elements, allantoin or its derivative and/or one or more auxiliaries; or the mixing up of certain amount of choline or its derivative or its agriculturally applicable salt with certain amount of mineral elements, allantoin or its derivative and/or auxiliaries, then further mixing with certain amount of agricultural carrier or diluent.

The composition may be used to accelerate photosynthesis in various plants and, to inhibit photorespiration thus greatly increasing the efficiency of photosynthesis. The composition also features plasticity/drought resistant of plants.

The following programs and examples of implementation serve only to illustrate the present invention without limiting its range.

In a prefered scheme of the invention chlorocholine was put into an enamel jar added with suitable amount of water under stirring to make a solution of 58-73% in concentration, then added with certain amount of mineral elements and allantoin to desolve them with stirring to end concentrations of 1-5% and 1.00-2.50% respectively. Acidity of the final solution was adjusted to neutral. Finally a brown homogeneous solution obtained is the photosynthetic accelerator.

The composition of the invention prepared as above was diluted to 1500-2000 times, then was introduced into the cytoplasm of plant leaf cells by vacuum infiltration or by direct spray. After a period of time, the isolated chloroplats were extracted and tested, and found that compared with the control, Hill reactivity increased by 1.16-1.23 fold; ATPase activity-1.6-1.9 fold; activity of RuBP carboxylase-1.2-1.7 fold; G-3-P dehydrogenase activity increased by 0.5-0.8 fold; protein content increased by 29.6-32.4%; chlorophyll content increased by 26.8-34.4%; rate of photosynthesis increased by 44-68%; and the glycolate oxidase, the key enzyme of photorespiration was inhibited by 40-50%. After spraying with the composition of the present invention in various concentrations at different growth stages of various plants, all the above indexes were imporved more or less. It indicates that the composition of the present invention showed a clear activation on the key enzyme

of photosynthesis, ATP enzyme, RuBP carboxylase and G-3-P dehydrogenase and a strong inhibition on the key enzyme of photorespiration and, glycolate oxidase. After international computer hook up retrieval there have not been such reports about the study on mechanism at home or abroad. Through the yield tests on the field crops sprayed with the photosynthetic accelerator, the effect in yield increase was more significant than other agents for increasing in yield accelerators: in field crops the yield over control 15.5-25%, in cotton 16.2-22%, in vegetables 30-60%, and in fruit trees 26-35%. All these data indicate that the present invention is an advanced measure for a fundemantal resolution of promoting photosynthesis to direct agricultural practice for bumper yields; furthermore it fills up the gap for years of utilization of chemical reagents in practice for hight yield, instead of only in theoretical studies about photosynthesis due to high reagent cost.

The practical examples of the invention are illustrated in the following aspects.

(1) The influence of chlorocholine on pure ATPase and RuBP carboxylase in winter wheat: Prepare 0 ppm, 200 ppm, 500 ppm and 1000 ppm chlorocholine solutions; introduce them into plant leaves by vacuum infiltration; then determine the change in activities of these enzymes in leaf extraction. The methods of preparation, determination and results are described in application example 1.

(2) The effects of chlorocholine on RuBP carboxylase, G-3-P dehydrogenase, chlorophyll content, protein content and rate of photosynthesis for winter wheat in different growth periods.

Prepare 0 ppm, 200 ppm, 500 ppm and 1000 ppm chlorocholine solutions, and spray the plants at different growth periods; afterwards extract and determine the changes of RuBP carboxylase and G-3-P dehydrogenase activities, chlorophyll content, protein content and photosynthetic rate. Please see application example 2 for details.

(3) Studies on the effects of chlorocholine on increasing yield in different crops.

Prepare 0, 20, 50, 100, 200, 500, 1000 and 2000 ppm chlorocholine solution; spray different crops at different growth periods; inspect the situations of increase in yields of various crops by chlorocholine. Please see application examples 4, 5, 6 for results.

(4) The inhibition of glycolate oxidase, the key enzyme of photorespiration, by allantoin.

Allantoin a constituent in the composition of the invention acts as the inhibitor of photorespiration, i.e. inhibits the activity of the key enzymeglycolate oxydase, and consequently part of the photorespiration. Plants with strong photorespiration (especially the C_3 plants, i.e. wheat, rice, cotton, vegetables etc.) will

consume part of the photosynthetic product in photorespiration; therefore they produce less than plants with weak photorespiration. When photorespiration is suppressed by around 30-50%; the yield will increase by 4-10%.

In the invention, it is indicated that the addition of allantoin in different concentration inhibits the activity of glycolate oxydase to different extents and hence promotes the accumulation of photosynthetic products and the yields. Please see application example 3 for details.

(5) The effect of the composition of the invention on various crop plants for increasing production.

Prepare the photosynthetic accelerator solutions of different concentration and make spray experiments on wheat, rice, cotton, garlic, spinach, celery, cabbage, cucumber, grape, pear etc. Its effects an increasing yields are significant. See the results in application examples 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16.

Example 1

The influence of chlorocholine on the enzymatic activities of pure ATPase and pure RuBP carboxylase in wheat.

Chlorocholine was determined by Hitachi 260-50 type infrared spectrometer; the spectrogram and standard spectrum coincided

completely; its content was determined by anhydrorus titration and identified by gas chromatography (SP-2305E); the purity is 99.99%. 0 ppm, 200 ppm and 500 ppm chlorocholine were prepared.

RuBP, NADH (coenzyme I), phosphoinositide and glucosan gel (Sephadex) for experiment all are the products from Sigma Chemical Company U.S.A.

The preparation and determination of RuBP carboxylase were carried out as described by Wu Guang-yao (Journal of Plant Physiology, 6, 3(1980). Samples in triplicate each 50g of wheat leaves were taken and washed then wiped out water; and immersed separately in 200 ppm and 500 ppm chlorocholine solutions, warmed up to 30°C for 10 minutes; one duplicate submerged in distilled water (0 ppm) as control. All the three materials were subjected to vacuum infiltration (750 mm Hg) for 40 minutes till the leaves turned transparent then transferred into refrigerator for an hour.. Equal amount of 0.5 mol/L phosphate buffer (pH 7.4, containing 1 mmol EDTA and 0.5 mmol mercaptoethanol) was added to each sample and the samples were crushed up in homogenizor. The homogenates were filtered with double nylon cloth. The filtrates were regulated to pH 6.4 and incubated at 60°C water both for 10 minutes, then kept in ice bath at once. The supernatant liquids were centrifugalized in cryocentrifuge (zuko RS-20 type) at 10500 x g for 10 minutes, after 35% saturation and 55% saturation $(NH_4)_2SO_4$ salting out centrifugalized at 10500 x g once more. The pellets were resuspended in phosphate buffer (at pH 7, containing 0.1 mmol EDTA

and 10 mmol mercapto ethanol). The resuspended solutions passed through Sephadex G-50 chromatographic column to G-200 chromatographic column. The eluents after chromatography were subjected to salting out in $(NH_4)_2SO_4$ at 60% saturation, then centrifugalized for 10 minutes. The pellets were resuspended in 0.01 mol/L Tris-HCl buffer (pH 7.8). Lastly passing through Sephadex G-25 for desalting the pure enzyme-RuBP carboxylase was obtained. The enzyme in polyacrylamide gel electrophoresis expressed in a single band. With UV-265 spectrophotomer (Shimadzu Co.,) and according to Racker's spectrophotometric coupled with NADH oxydation method to determine the activity of enzymes (Racker 1962): the multyenzyme system assay consisted of 90 mmol/L Tris HCl buffer (including 12 mmol $MgCl_2$, 0.4 mmol EDTA, pH 7.8); 50 mmol ATP; 25 mmol RuBP; 3 mmol NADH; 50 mmol phosphocreatine; 20 mmol $NaHCO_3$, phosphoglyceric acid kinase, glyceraldehyde phosphate dehydrogenase, RuBP carboxylase 0.1 ml; the amount of NADH oxydized in μmol per minute served as the activity of RuBP carboxylase.

the results are shown in Fig. 1. In the figure it is shown that chlorocholine significantly promoted the activity of RuBP carboxylase activity by increasing 1.7 fold over control.

The test for the activity of ATPase on grana lamellae of chloroplasts followed the method by Huang Zhuo-hui ("Research Methods of Plant Physiology and Biochemistry" Shanghai Science Publishing House, 1985) and the enzymatic protein content test was

carried on according to Lowry's Folin-phenol method (J. Bio. Chem. 193:263, 1951).

The method of preparation and test for activity of ATPase on the grana lamellae of chloroplasts: 10 g of wheat leaves were taken, washed and wiped dry and added to chlorocholine 0, 200, 500 ppm solutions respectively; the 0 ppm solution served as control. The solution were introduced into wheat leaves by vacuum infiltration for 40 minutes until the leaves tuned transparent. The preparations of the activator and reactant solutions for the Mg-ATPase on thylakoid membranes were as follows:

Activator solution		Reactant solution	
Tris-HCl,pH8.0,	0.25mol/L 0.2 ml	Tris-HCl,pH8.0,	0.5 mol/L 0.1ml
NaCl	0.5 mol/L 0.2 ml	$MgCl_2$	0.05mol/L 0.1ml
$MgCl_2$	0.05mol/L 0.2 ml	ATP	50 mmol/L 0.1ml
DTT	50 mmol/L 0.2 ml	H O	0.2ml
PMS	0.5mmol/L 0.2 ml		

Discarding the insoluble coarse debris from the homogenized leaf samples, the chloroplasts were resuspended in Tris-HCl (pH7.8). Adding 1 ml chloroplast suspensions (chlorophyll content about 0.1mg/ml) and 1 ml activator solution, the photoactivation carried on for 6 minutes. Three test tubes were used for each group of treatment, and added with the activated chloroplast suspension

0.5ml, and 0.5ml reactent solution, incubated in 37°C water bath for 10 minutes (one tube kept in ice bath as control), then 0.1ml 20% trichloroacetic acid was added to each test tube to stop the reaction. The samples were centrifugalized and the supernatant in each tube was collected about 0.3-0.5 ml for the determination of the inorganic phosphorus/hydrolyzed from ATP.

The determination of inorganic phospherus 2.5 ml of distilled water was added to 0.5ml supernatant in each tube, agitated well then 2ml ferrous sulphate-ammonium molybdate reagent was added. The color reaction appeared after 1 minute under room temperature. The quantity of phosphorus was determined by colorimetric method at 660mn, with the inorganic phosphorus standard curve made by the solutions of 0.001M Na HP04 in 20% trichloroacetic acid-ammonium molybdate at different concentrations. The abscissa was the concentration of inorganic phosphorus determined with UV-256 spectrophotometer at 660 Onm and the ordinate represented the optical density. With these data the standard curve was traced. The enzyme activity was calculated according to following formula. MP X V/V X 1000/chlorophyll X 60/t = MP /n+g chlorophyll hr (V and V the volume of reactant samples).

The results of the experiment are shown in the following table (Table 1).

Table 1 The influence of chlorocholine on the ATPase in the Chloroplasts of Winter Wheat

chlorocholine (ppm)	Activity of ATPase (mol pi, mg chl h)	
0 (control)	54,492	100
200	89.243	164
500	104,224	191

The data in Table 1 indicates that chlorocholine showed a clear promotion on the activity of ATPase and 500ppm chlorochcline solution helps the activity of ATPase increase to 1.9 fold over the control.

Example 2

The influence of chlorocholine on the RuBP carboxylase and G-3-P debydrogenase activities, chlorophyll content, protein content and photosynthetic rate of winter wheat at different growth phases.

The variety of winter wheat for this investigation was 7225, and the soil fertility of the experimental field was homogeneous. The size of experiment plots was 6m X18m, and the application of compound fertilizer to each plot amounted to 25kg/mu. Seed rate to each plot was 7.5g, amounted to 8.4kg/mu. At seedling, booting, and flowering and milk ripe stages, samples of leaves were collected ten days after spraying chlorocholine solutions of 0 50, 100, 200ppm in concentration.

(1) The preparation and determination of G-3-P dehydrogenase: At the respective growth phase, the first four leaves from flag leaf downward were picked and collected as sample for test. Every treatment group contained 10g of leaves, precooled, peeled off the midribs, cut into fine pieces,and then added with 30ml of extraction solution precooled to 0.°C (0.05 mol Tris-HCl buffer solution containing 0.35 mol sorbitol, 0.002 mol EDTA, 0.1% mercaptoethanol, at pH7.8) , followed with crushing in high speed homogenizer. The homogenates were filtered through double nylon cloth filters (mesh 100). The supernatant was centrifugalized in cryocentrifuge (Seiko RS-20type) at 600 x g, 4500 x g and 9200 x g respectively. The crude G-3-P enzyme solution was determined for the activity of G-3-P dehydrogenase with Racker spectrophotometer. Th enzyme reactant system consisted of Tris Hcl 80μmol, $MgCl_2$ 10μmol (pH8.4), DTT 2.5 μmol, ATP-Na 5μmol, NADPH 0,2μmol, 0.3ml G-3-P dehydrogenase crude enzyme extraction. All the ingredients were warmed up in 30°C respectively for 10 minutes, then mixed up and warmed up again for 15 minutes; the reactions started. In the control distilled water was used instead of G-3-Pase.

The results of determination is shown in Fig 2. The result in Fig. 2 indicates the increase in activity of G-3-P dehydrogenase after the spray of chlorocholine in different cencentrations.

(2) The preparation and determination of RuBP carboxylase: 3 wheat leaves from the flag leaf downward were taken at different growth

phases. Every sample contained 5g of wheat leaf precooled, with midribs peeled off, cut into samll pieces. 12ml extract solution precooled to 0°C (0.1mol/L Tris-HCl buffer solution, containing 12 mmol $MgCl_2$, 0.36 mmol EDTA and 5 mmol mercaptoethanol, pH7.8) was added to each sample. The mixture was crushed with quartz sand in a precooled mertar into homogenate. The homogenate was filtered through double nylon cloth filter (mesh 100). The supernatant was centrafualized in high speed cryocentrifuge (Shimadzu Co, IRS-20 type) at 10500 x g for three times each 15 minutes. After centrifugation the centrifgalized fluid was subjected to fractional extraction with 35-60% $(NH_4)_2SO_4$. After the extraction the RuBP carboxylase crude enzyme solution was prepared. Before the determination of enzymatic activity, the crude enzyme solution had to be desalted through Sephadex G25 with 0.1 mol/L Tris HCl eluant. The activity of RuBP carboxylase was determined with racker spectrophotometric method.

The results of the determination are shown in Fig. 3. In Fig 3 it is illustrated that after sprays of chlorocholine at different concentrations the activity of RuBP carboxylase increases over control by 2.1 times.

(3) The determination of chlorophyll and protein contents: The chlorocholine solutions at the concentrations cited above were sprayed on the wheat plants at respective growth phases. The protein contents at four growth phases increased as compared with control; at booting stage the protein content was the highest by

32.4% over control, and at milk ripe stage the protein content increased by 29.6% over control.

The chlorophyll content also showed a significant increase over control at every growth phase: at milk ripe stage it over-passed control by 34.4%; at booting stage 26.8%; even at seedling stage 15.4% over control, therefore after 5-8 days sprayed with 500ppm chlorocholine solution, the leaf color of the plants at different growth phases turned sigrificantly greener, even visible to the naked eyes.

(4) The influence of chlorocholine on the rate of photosynthesis of winter wheat

At flowering stage the wheat leaves were sprayed with 200ppm and 500ppm chlorocholine solution, then tested for the photosynthetic rate of the flag leaves with infrared CO analyser. The results showed a tendency of increase in photosynthetic rate with the increase of chlorocholine concentration. The treatment of 500ppm chlorocholine solution showed a 68.79% increase over control, while that of 200ppm solution 51.28%.

The determination of chlorophyll content was carried out as described by Arnon's method (plant Physiol., 24(1)1-15,1949).

The extraction and determination of RuBP carboxylase at different growth periods of winter wheat followed Feng Fusheng's method (Newsletter of Plant Physiology, 1986(6):20-22).

The extraction and determination of G-3-P dehydrogenase at different growth phases were done according to Feng Fushen's method (Newsletter of Plant Physiology, 1984(4):25-27).

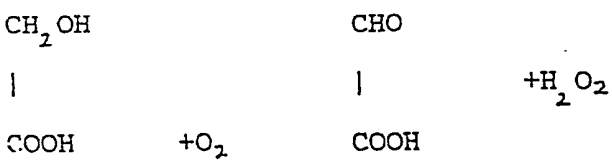
The determination of photosynthetic rate is measured with infrared CO_2 analyser according to Sestak et al. (infrared Gas Analysers and Other Physical Analysers, 1971).

Example 3

Inhibition experiment of allantoin to photorespiration, key enzyme glycolate oxidase

(1) The preparation and spray of allantoin 0,10,50,100,200,500 ppm solutions of allantoin were prepared and sprayed onto wheat plants at booting and milk ripe stages in six groups; samples of these groups were collected ten days thereafter.

(2) The extraction and determination of glycolate oxidase: Glycolate oxidase plays an important role in photorespiration of plants; it can oxidize glycollic acid into glyoxalic acid and produce hydrogen peroxide.



2,6 dichlorophenol indol ol is used here as hydrogen receptor, which received the hydrogen released after the oxidation of glycollic acid and was reduced itself; then the original blue color of dichlorophenol indol - ol disappears. The reaction is as follows.

Oxidized form dichlorophenol indolol (blue colored)

+

Glycollic acid

Réduced form dichlorophenol indolol (colorless)

+

Glyoxalic acid

Because of the presence of indophenol oxydase in the crude extraction of glycolate oxydase, which can induce the oxidation of dichlorophenol indophenol and consequently influence the determination; therefore it is necessary to add KCN, which is effective in suppressing indophenol oxidase and ineffective to glycolate oxidase.

1. The preparation of crude extract of glycolate oxidase

Samples of 10g of wheat leaves from the groups cited above were collected and washed; after adding 30ml water crushed up in ice cooled mortar, and filtered through 4 layers of gauze.. The filtrate was centrifugalized in 1000 x g cryocentrifuge at for 15 minutes. With sediment removed, the supernatant was adjusted to pH 5.4 with 10% acetic acid and centrifugalized again at 3500-4000 x g for 15-20 minutes to get rid of miscellaneous proteins. To each 10ml crude extraction, 1.4g $(NH_4)_2SO_4$ was added in order to reach 20% saturation and stirred incessantly for 30 minutes, centrifugalized again to remove precipitate. 0.7g of $(NH_4)_2SO_4$ is added to the supernatant to attain 30% saturation, then centrifugalized once more at 3500-4000 x g for 20 minutes. The pellet was dissolved in 0.2mol, pH8.0 phosphate buffer. The solution was the crude glycolate oxidase.

2. The determination of glycolate oxidase activity

To the above 6 groups of treatment and the blank of colorimetry, altogether 7 groups in separate test tubes, 0.6ml KCN(0.01 mol/L), 0.04 mol/L potassium glycolate 0.5ml, 0.5ml glycolate oxidase solution diluted to 5 times, 1 ml phosphate buffer were added. Except the blank of colorimetry, into the six groups 0.3ml 2.6-dichlorophenol indophenol was added to make up the volume to 3ml, then quickly transferred into cuvettes with 1cm light path, measured in 620nm visible ultraviolet spectrophotometer (Hitachi, UV-265 type). In the blank test tube, buffer solution was used instead of 2.6-dichlorophenol indophenol.

The amount of enzyme required to reduce 0.01 optical density was set as 1 unit. Let the fresh weight of material required be the activity of the enzyme.

3. The results of inhibition of glycollate oxidase activity by allantoin.

Allantoin concentration (ppm)	0	10	50	100	200	500
Booting stage	0	-0.9%	-8.2%	-18.5%	-49.4%	-50.1%
Milk ripe stage	0	-1.0%	-8.8%	-17.6%	-51.3%	-49.8%

From the above table allantoin shows significant inhibition on glycolate oxidase; 200ppm allantoin reduces the activity to about 50% at 2 main growth stages of wheat, therefore the photorespiration cannot carry on. About 50% photosynthetic product ribulose 1,5-diphosphate remained unconsumed in photorespiration. Theoretically the rate of increase in production by 200ppm allantoin would be around 10%.

Example 4

The effect of chlorocholine on the increase of yield in winter wheat

Since 1988 the solution of chlorocholine in different concentration and accompanied with different minor elements has been extensively

applied in spray experiments from small plots to large areas for 3 years, all the experiments ended in success.

Sprayed simply with 0, 100, 200, 500 or 1000ppm chlorocholine solution, the wheat plants increased in yield to various extents, especially the sprays at booting and milk ripe stage promoted the yield significantly, the increase in yield ranged between 10.6-11.8%. 500ppm chlorocholine solution accompanied with mineral elements and sprayed at booting and milk ripe stage promoted wheat yield around 12.0-15.1%.

Application Example 5

The experiment on the effect of chlorocholine on cotton yield

Spraying once at flower bud stage and boll setting stage of cotton with 200, 500ppm chlorocholine solution (0 ppm solution as control) showed relatively good effect with yield increasing about 10%; while accompanied with mineral elements in the spray it reduced the rate of flower and boll drop by 20% and promoted cotton yield 12.2-14.8%, in same filed blocks the ginned cotton by 20kg.

Example 6

The experiments on the effect of chlorocholine on the increase in production of celery and spinach.

The celery and spinach both are leaf vegetables with short growth period (about 60 days). Spraying with chlocholine accelerated the growth of stems and leaves and hastened maturity because of the increase of sugar, protein and chlorophyll contents after spraying 200, 500ppm chlorocholine solution ~~and sprayed~~ (0 ppm chlorocholine solution as control) the celery might increase in yield by 22% and spinach 19.2%. With additon of minor elements celery yield increased by 28% and spinach 25%. 100ppm chlorocholine solution also showed an increase in yield about 10%.

Application Example 7

The effect of the composition of the invention (chlorocholine, minor elements and allantoin) on the increase in yield of winter wheat.

The constituents of the composition in percentage

chlorocholine	63%
minor elements	2.85%
allantoin	1.23%
soda	0.22%
water	32.7%

The above solution was diluted to 1500-2000 times then sprayed upon wheat plants once at booting and milk ripe stages respectively (neutral or weak acidic pesticides might be added). The time of spraying was before 10 O'clock A.M. or after 4 O'clock P.M. If it rained within 6 hours after spraying, it is necessary to repeat

spraying. 3-5 days after spraying with the photosynthesis accelerator the leaf color of the treated plants was much greener than the control due to the increase in chlorophyll content and the stems became stouter than those of control 10 days after such treatment. At the later period of wheat growth, dry and hot wind prevails in northern China; because chlorocholine functions to increase draught resistance in plants, the plants sprayed showed more resistant to drought than the control. Their filling up of seeds went on normally, yellow ripe stage carried on with about 2/3 of leaves and stalks remained green in color, especially the flag leaves remained not dried up and green still and could carry on normal photosynthesis, so that the grains were plump and full and the number of grains per spike increased by 1-3 grains (the number of spikes per mu averages 400,000, after spay the number of grain might increase by 40,000-120,000 grains/mu)), the thousand grain weight increased by 2-4g. Generally at the later growth period the roots of wheat plants become dried up and die early. When sprayed with the photosynthetic accelerator of the invention the roots of plants remained alive but not late maturing. In the last two years experiments, the results showed a significant increase in yield rate as high as 15.8-22.4%; in typical wheat field there was the example of increasing yield by 71.5kg/mu. In Fig. 4, the left side is wheat plants sprayed while the right side is control.

Example 8

The experiments on the effect of the photosynthetic accelerator of the invention on the increase of of yield

The solution was prepared and diluted according to the procedure in application example 7. Diluted solution of 200 and 500ppm (stock solution) were sprayed once at booting and milk ripe stages respectively 30kg of diluted solution was needed for spraying the leafage of one mu of rice plants. 4-8 day after spraying the leaves turned greener than the control, the stalks became stouter, the shooting came out neatly. Because the number of effective spikelets increased after the spray of photosynthetic accelerator at booting stage, the number of grains per ear might increase 2-4.The average number of ear per mu was 250,000; but the sprayed grain with accelerator might increase by about 30,000 ears over the control. Spraying with photosynthetic accelerator at milk ripe stage premoted the rate of filling up of grains, and the thousand grain might overweight the control by 3-5g; especially in rainy weather the rice plants sprayed with photosynthetic accelerator showed a higher photosynthetic rate over control, and could perform a more or less normal photosynthesis. Because of the stouter stalks of the treated plants, the resistance to lodging increased. From the experiments in recent years, it proves that the effect of the photosynthetic accelerator of the invention on the yield of rice is more significant than that in winter wheat and the average rate of increase was 16.8-25%, in typical rice field yield increase might be as high as 85kg/mu.

Example 9

The experiment on the effect of photosynthetic accelerator to yield increase in cotton.

The solution was prepared and diluted according to the procedure in application example 7. The plants were sprayed two times, one at the appearance of flower buds and the other at early bollforming stage. 8-10 days after spraying, the leaf color turned dark greener than the control, the stems became stouter and the plants showed stuntedness. Because the photosynthetic accelerator might enhance the resistance to draught, the drop of flower buds and bolls due to draught could be alleviated, and at the same time the increase of the content of photosynthetic products improved the carbon-to-nitrogen ratio (C/N), misproportion of which is also one of the important factors causing flower/boll drop. After being sprayed with photosynthetic accelerator, the rate of flower/boll drop reduced to about 20-40% over control (in ordinary cotton filed may be as high as 60%). Cotton is a typical C plant with very prominent photorespiration. The allantoin contained in the accelerator may inhibit part of the photorespiration, thus increasing the yield accordingly. Over the past three years in the fields sprayed with photosynthetic accelerator, the reduction of summer boll drop was a striking character with yield increase up to 16.2-22% and 30kg grinned cotton/mu in typical cotton fields achieved.

Example 10

The experiment on the effect of photosynthetic accelerator to the increase in yield of garlic

The solution was prepared and diluted according to application example 7. Before and after shooting of garlic the plants were sprayed each once. Because of the increasing photosynthetic rate, the leaves of garlic turned dark green, shooting enhanced and the sheet was stouter about 1/3 in thickness over control 3-5 days after spraying. Because the chlorocholine contained in the photosynthetic accelerator not only promoted the increase of photosynthetic products, but also accelerated the transportation and storage of food and minor elements B etc. therein may also enhance food transportation; the production of garlic scapes might increase by about 15% and hence the garlic bulbs by about 50% or more. In Fig 5. On the left is the garlic sprayed with the accelerator and the control is on the right.

Example 11

The experiment on the effect of the photosynthetic accelerator to the yield of Chinese cabbage

The method of preparation and dilution of spray solution was the same as in application example 7. At seedling stage and the middle and late growth stages the plants were sprayed once respectively.

Chinese cabbage is a common vegetable both in Northern and Southern China; in the South Chinese cabbage is one of the vegetables available throughout the year. Chinese cabbage has a short growth period. As the photosynthetic accelerator is toxic/pollution free,it can be applied at later growth period. Sprayed at seedling stage, the growth of the plants accelerated. Owing to the increase in photosynthetic product and chlorophyll, the Stems and leaves became tender and thicker, leaf color bright green and the plant type was uniform. Under the rainy weather in the South, the roots did not rot and matured 3-5 days earlier than the control. In Fig. 6, A is the Chinese cabbage sprayed with the accelerater and B, the control.

Example 12

The experiment on the effect of the photosynthetic accelerator to grape yield.

The preparation of the spray solution is the same as the procedure in application example 7 and diluted before use. At the ealier stage of flowering, young fruit stage and medium fruit stage, the plants were sprayed once respectively. As a result of the increase of photosynthetic product enhanced by the photosynthetic accelerator at the early growth period and the increase growth hormone by Zn in the minor elements component, the development of flower buds carried on well, the number of flower clusters increased and the grape plant produced more berries. The

photosynthetic accelerator promoted the input of sugar at young and medium fruit stages, so that the sprays enhanced early maturity. In the experiment the rate of increase in fruit production after spraying with 200ppm solution was by 28-32%, with 500ppm solution by 30-35% and it helped the fruits turn violet (mature) earlier by about 10-20 days.

On the basis of the formula of the invention, the effect on yield increase for various form crops were as follows:

The rate of yield Increase of Various Crops (%)

Crops	Chlorocholine	Chlorocholine + Mineral elements	Allantoin	chlorocholine + Mineral elements + Allantoin
Wheat, Millet, Rice	10.6-11.8	12.0-15.1	4-6	15.5-25
Maize, Sooghum, Cotton	9.2-11.5			
12.2-14.83				
4-8	16.2-22			
Leaf vegetables	18-22	24-28	6-10	30-45
Fruit vegetables	24-28	26-40	8-10	42-60
Fruit trees	15-18	20-22	4-6	26-35

Application Example 13

The experiment on the effect of the photosynthetic accelerator used as seed dressing for wheat and cucumber

One day before wheat and cucumber seedings 1.6ml of the photosynthetic accelerator prepared as the procedure in application example 7 was diluted with 60 ml fresh water to dress the seeds for one mu (10kg of wheat grains or 1.5kg of cucumber seeds). The germination rate of treated wheat seeds was higher by 8.5% over control (germination rate of the control was 91%, the dressed seeds 99.5%). The treated cucumber's germination rate was 5.1% higher than the control (the germination rate of the control 91.5%, that of the treated seeds 95.6%). The rate of increase in yield of the treated wheat was 12.2% higher than the control; in case of cucumber the increase in yield was 26.4% higher than the control.

Example 14

The experiment on the effect of the photosynthetic accelerator combined with pesticides on wheat yield.

The solution of the composition of the invention prepared and diluted according to the procedure in application example 7 and added with methamidophos in 500 times dilution was used to spray wheat at booting stage and milk ripe stage respectively. The plants sprayed with photosynthetic accelerator and methamidophos mixture yielded more by 3% over the accelerator without methamidophos and over the control (sprayed without photosynthetic

accelerator and methamidophos) by 22.4%. The photosynthetic accelerator and methamidophos did not interfere with each other in functioning when sprayed together.

Example 15

The experiment on yield-increase effect of the application of photosynthetic accelerator in powder form to the root tubers of sweet potato.

70% chlorocholine, 3% minor elements and 1.23% allantoin solution were added to bentonite to make a dressing powder containing 25% chlorocholine. 0.5kg of this powder was mixed up with 9.5kg sail; the mixture was dressed to the basal parts of sweet potato plants in the middle of the growth period, and well watered with the result that the root tubers were much bigger, yieded more 40% over the control.

Example 16

The experiment on yield-increase effect in cucumber sprayed by photosynthetic accelerator with electric atomizer

The composition of this invention was prepared as described in application example 7; the resulting solution was diluted and applied at early period of formation of female flower , young fruit and medium fruit stages once respectively. Because the sprays by

electroatomizer was homogeneous, the cucumber (especially green house cucumber) fruiting rate was 10-18% more than the control. Besides, the fruits were bigger and tender. As a result the rate of yield increase was 40% over the control; in some examples for a 4/10 mu area in green house (plastic green house), nearly a hundred catties of cucumber might be gathered daily.

CLAIMS:

1. An agricultural composition which promotes the photosynthesis of plants comprising an effective amount of choline or its derivatives or agriculturally acceptable salts and an agriculturally acceptable carrier.

2. An agricultural composition according to claim 1, which further comprises an effective amount of mineral elements.

3. An agricultural composition according to claim 1 or claim 2, which further comprises an effective amount of allantoin or its derivatives.

4. An agricultural composition according to any one of the preceding claims, which further comrpises one or more kinds of auxiliaries.

5. An agricultural composition according to any one of the preceding claims, wherein the content of said choline and/or its derivatives and/or salts is 0.001-99%, mineral elements 0-30%, allantoin and/or its derivatives 0-99% and auxiliaries 0-25%.

6. An agricultural composition according to any one of the preceding claims, wherein said choline or its derivatives and salts are cholinehydrochloride, cholinehydrobromide, cholinehydroiodide, choline phosphate, choline hydrogen phosphate, choline sulphate, choline hydrosulphate, choline bicarbonate, choline hydrosulfite, choline tartrate, choline hydroxide or phosphatidyl choline.

7. An agricultural composition according to any one of claims 2 to 6, wherein the mineral elements are selected from B, Mg, Zn, Mo, S, Mn, Cu, Fe, K, Ca, P, I, Na, Si or a mixture of two or more of the above elements.

8. An agricultural composition according to any one of the preceding claims, wherein the agriculturally acceptable carrier is water.

9. An agricultural composition according to any one of claims 1 to 7, wherein the agriculturally acceptable carrier is a conventional solid carrier.

10. An agricultural composition according to claim 9, wherein the conventional solid carrier is selected from the group consisting of kaolin, bentonite, white diatomite, calcium carbonate, talc, magnesium oxide powder, bleaching earth, gypsum, diatomite and pottery clay.

11. An agricultural composition according to claim 1, substantially as hereinbefore described with reference to any one of the Examples.

12. A process for preparing an agricultural composition according to any one of claims 1 to 11, which comprises mixing choline or its derivatives or agriculturally acceptable salts with an agricultural carrier and optionally adding mineral elements, allantoin or its derivatives and/or auxiliaries.

13. A process for preparing an agricultural composition according to claim 12, substantially as hereinbefore described with reference to any one of the Examples.

14. A process for preparing an agricultural composition according to any one of claims 1 to 11, which comprises mixing first choline or its derivatives or agriculturally acceptable salts with mineral elements, allantoin or its derivatives and/or auxiliaries, and then blending with an agricultural carrier.

15. A process for preparing an agricultural composition according to claim 14, substantially as hereinbefore described with reference to any one of the Examples.

Patents Act 1977
Examiner's report to the Comptroller under Section 17
(The Search report)

Application number
GB 9315734.5

Relevant Technical Fields

(i) UK Cl (Ed.L) C1B

(ii) Int Cl (Ed.5) A01N

Search Examiner
R HONEYWOOD

Date of completion of Search
19 OCTOBER 1993

Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASE: WPI

Documents considered relevant following a search in respect of Claims :-
1-15

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	GB 2134505 A	(VEB CHEM BITTERFELD)	1 at least
X	GB 2079605 A	(SAMPSON)	1 at least
X	GB 2059412 A	(ISRAEL MIN OF AGRIC)	1 at least
X	GB 2052260 A	(SAMPSON)	1 at least
X	EP 0220514 A	(MITSUBISHI GAS CHEM KK)	1 at least
X	EP 0190561 A	(BAYER AG)	1 at least
X	EP 0167776 A	(HOECHST AG)	1 at least
X	EP 0146017 A	(MITSUBISHI GAS CHEM KK)	1 at least
X	EP 0009696 A	(BAYER AG)	1 at least

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).