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Effect of PBAT Biodegradable Mulch Film Extract on Seed Germination and Seedlings Metabolism of Tobacco

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Abstract: Poly(butylene adipate-co-terephthalate) biodegradable mulch film (PBAT-BMF) is gradually applied in agricultural production, but its potential ecological risks have not been studied so far. In this paper, methyl tert-butyl ether (MTBE) was used to extract organic compounds from PBAT-BMF to evaluate its effects on tobacco seed germination and seedling metabolism. The results showed that six organic compounds were found in the extract of PBAT-BMF, among which the content of diisopropylphenyl isocyanate was the highest at 557.27 μg g^{-1}. A germination test showed that the extract scan inhibited the germination of tobacco seeds. The germination time was 1 d later than the control(CK), and the germination percentage and germination energy were non-significantly decreased by 2.50% and 1.00%. In contrast, the extracts significantly affected the root length, and seedlings height ($p < 0.01$), decreased by 0.24 cm and 0.28 cm, respectively. A metabolic analysis revealed that the extracts have a certain stress effect on tobacco seedlings and showed an up-regulating effect on soluble sugar, critical organic acid, biogenic amine, and down-regulating alkaloid, which indicated that the carbon and nitrogen metabolism pathway of the tricarboxylic acid cycle and alkaloid synthesis were disturbed. These results indicated that organic compounds extracted from PBAT-BMF had stress effects on germination and the growth of tobacco seeds, which significantly changed the metabolism pathway. This study can provide a theoretical basis for the development and application of PBAT-BMF.

Keywords: PBAT biodegradable mulch film; seed germination; extracts; metabolites; metabolism pathway

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1. Introduction

Film mulching can improve the microclimate of the soil surface by increasing the reflectivity of sunlight and the resistance of air flow [1] so as to improve the net photosynthetic rate of crop leaves [2] and realize the increase of yield and the efficiency of crops. However, with the continuous increase of the durable years and quantity of mulch film, the residue in the soil is becoming quite significant [3]. The residual mulching film (RMF) will not only destroy soil structure and affect soil permeability, but also hinder the migration of water and nutrients in the soil to crops [4]. This will be detrimental to crop growth and development, resulting in crop yield reduction [5], and eventually cause serious "white pollution" to the agricultural environment and ecological security [6].

In order to reduce and prevent residual pollution of mulch film, biodegradable mulch film came into being with the development of biodegradable materials, and had been gradually applied in agricultural production as an ideal measure to solve the

worldwide problem of "white pollution" [7]. The bacterium of *Pseudomonas putida* identified from soil particles directly attached to the surface of bioplastics were responsible for the biodegradation [8]. Thermoplastic biodegradable plastic poly (butylene adipate-co-terephthalate)(PBAT), as a kind of polyester biodegradable material, was a copolymer composed of butylene adipate (PBA) and butylene terephthalate (PBT) [9,10]. It is widely used as a raw material for biodegradable mulch films due to its excellent softness and ductility. [11]. However, compared with traditional plastics, the mechanical properties of PBAT are still an important factor limiting its wide application. Many studies aim to increase the mechanical properties while synthesizing PBAT [12,13], and to improve the comprehensive properties of PBAT substrates by blending them with other polymers [14,15].

At present, some studies have proved that the effect of biodegradable plastic on plants may be much more serious than that of low-density polyethylene (LDPE). Wang reported that 10% polylactic acid (PLA) significantly inhibited photosynthesis and reduced maize biomass [16]. Zhou indicated found that 10% poly (3-hydroxybutyric acid-co-3- hydroxy valerate) (PHBV) caused wheat plants death after 25 days [17]. However, whether PBAT biodegradable mulch film (PBAT-BMF) is also detrimental to plants and microbiome systems is an open question [18,19]. In addition, some studies suggest that the organic additives of antioxidants, UV stabilizers, fillers, rubbers, lubricants, flame retardants, etc. need to be considered in discussing the ecological toxicity of degradable plastics. These additives contribute to an improvement of the mechanical properties of a polymer. Meanwhile, intermediate and final degradation product biodegradable plastics may also affect plant growth, although there is currently little concrete evidence [16]. Therefore, it is necessary to study whether additives or degradation products of PBAT-BMF have effects on plant growth and to clarify the main reasons for such effects.

China is the largest tobacco planting country in the world, with an annual tobacco planting area of about 1.4 million hm^2 [6]. Mulch film is a necessary production material for tobacco cultivation in China [20]. It has been widely used for many years, and almost all tobacco fields must be covered with mulch film [21]. In recent years, PBAT-BMF has been gradually used as a substitute for polyethylene mulch film on tobacco [22], but little research on its safety evaluation has been reported. Moreover, studies have shown that root elongation inhibition tests, seed germination tests and early growth tests of plant seedlings were the common methods used to evaluate the toxicity of substances [16–19]. In summary, these data allowed us to come up with the following hypothesis: the organic compounds may have an effect on plant germination and metabolism in the PBAT-BMF. To test this hypothesis, methyl tert-butyl ether (MTBE), which is highly volatile and had good extraction efficiency for low, medium and high polar compounds, was used as an extraction solvent to extract organic compounds from PBAT-BMF, and then the organic compounds were identified by GC-MS. Furthermore, tobacco seed germination and seedling experiments were carried out to evaluate the toxic effect of PBAT-BMF on plants so as to provide a reference for the development and application of biodegradable mulch film. This study provides a theoretical basis for the development and application of PBAT-BMF.

2. Materials and Methods

2.1. Test Materials and Reagents

The tobacco seed used in the experiment is K326 (naked seed), and the PBAT-BMF was purchased in the market of Guizhou Province. The specifications of the film were 120 cm wide and 0.008 mm thick. The main reagents included methyl tert-butyl ether (MTBE, chromatographic grade), PBAT extract quantitative internal standard (phenylethyl acetate solution 1.852 mg mL^{-1}), metabolite internal standard (adipic acid 10 mg mL^{-1}, phenylglucoside 8.04 mg mL^{-1}, n-valine 4.9 mg mL^{-1}, methanol and water 1:1 preparation), metabolite extract (methanol: trichloromethane: water = 2.5:1:1), methoxylamine hydrochloride,

pyridine, bis (trimethylsilyl) trifluoroacetamide, trimethylchlorosilane, etc., which were purchased from Sigma (St. Louis, MO, USA).

2.2. Experimental Process

5 g of PBAT-BMF and were weighed and put it in a conical bottle. According to the ratio of PBAT-BMF and MTBE at 1:20, the 100 mL MTBE was weighed and soaked in the conical bottle for 12 h at room temperature with dark condition, which was set up as the experimental group (T), and pure MTBE was used as control group (CK). MTBE was volatile and the conical bottle should be sealed in the process of soaking. After 12 h, the samples of the T and CK group were filtered with a 0.45 μm nylon membrane and transferred into a 100 mL distillation flask and dried with a rotary evaporator (RV10CS096) at 40 °C, respectively. The extract was extracted and placed for 24 h, then dissolved with 1-2 mL ethanol and 60 mL distilled water was added for the following experiments.

According to the rules for tobacco seeds (YC/T20-1994) [23], the experiment was performed with six replicates × two treatments (T and CK group). One hundred full and uniform tobacco seeds (naked seeds) were randomly selected and evenly sown in petri dishes (120 mm dia. × 20 mm height) covered with filter paper for the germination experiment. The temperature of the tissue culture room was 26 °C and the relative humidity was more than 85%. The dissolved extract was added daily by a weighing method to maintain constant weight to replenish the evaporated water. After being cultured in 12 h light and 12 h dark for 14 days, the seedlings of fresh tobacco leaves were taken for testing.

2.3. Relative Concentration of PBAT-BMF Extract

The PBAT-BMF extract were performed by dispersive liquid–liquid microextraction method with acetone/carbon tetrachloride mixture as dispersive solvent and extraction solvent and then determined with gas chromatography-mass spectrometry (GC-MS, Agilent 7890A-5975C). An HP-5 ms capillary column (60 m × 0.25 mm, 0.25 μm) was used for separation. An injector temperature of 280 °C, split injection, and a split ratio of 5:1 were used. The oven temperature was initially held at 60 °C for 2 min, raised at 15 °C min^{-1} to 230 °C for 5 min, followed by a second rate of 5 °C min^{-1} to 280 °C for 20 min and then the post-run was conducted by raising 3 °C min^{-1} to 290 °C for 5 min. The constant flow rate of helium carrier gas (99.999%) was maintained at 1 mL min^{-1}. The MS transfer line temperature was 280 °C. The ionization chamber and quadrupole temperatures were 230 °C and 150 °C, respectively. Electron impact mass spectrometric data in the range *m*/*z* 50–500 *amu* were collected using a scan rate of 3.99 s^{-1} with an ionization voltage of 70 eV, and the solvent delay was 7 min [24]. Each organic compound was identified by mass spectrometry with the Wiley08 and Nist14 MS libraries, and the relative concentration of the organic compound was quantitatively obtained by the internal standard method.

2.4. Determination of Germinative Energy and Germinative Percentage for Tobacco Seed

Combined with the rules for tobacco seeds (YC/T20-1994) [23]and Xiao's method [25], the number of germinated seeds was recorded every 24 h, and the germinative energy and germinative percentage of tobacco seedlings were calculated according to the standard of radicle elongation to seed length. Faster germinative speed and uniformity, indicating that the higher the seed vigor (Note: the number of germinated seeds was recorded every 24 h, and the day with the largest number of germination was the peak period of germination).

$$\text{germinative energy} = (\text{The number of seeds at the peak period of germination}/\text{Number of seeds tested}) \times 100\%$$

$$\text{germinative percentage} = (\text{The number of seeds germinated}/\text{Number of seeds tested}) \times 100\%$$

2.5. Determination of Radicle and Metabolites in Tobacco Seedling

On the 14th day, 10 seedlings were randomly selected from each petri dish, the radicles were cut off with a blade, and the seedling height and root length were measured. In addition, according to the determination method of Li [26], the metabolites of tobacco seedlings were determined and analyzed by GC-MS.

2.6. Data Processing

The study was conducted in petri dish experiments in complete randomized block design with six replicates in two groups. The variation analyses were undertaken with Microsoft Excel 2016 and DPS 17.10 software, principal component analysis (PCA) with euclidean distance as a similarity metric was carried out by SIMCA-P12. One-way ANOVA and multiple comparison was done by Duncan test. The assumption of homoscedasticity was tested using Levine's test. Logarithmic transformation of percentage data helps to meet a normal distribution. Other diagrams were plotted by OriginPro 9.0 software.

3. Results

3.1. Component Analysis of PBAT-BMF Extract

The component analysis of PBAT-BMF extract is shown in Table 1, including (Z)-9-octadecenoic acid amide, 2, 6-diisopropyl phenyl isocyanate, 2, 6-diisopropyl aniline, cyclodecane, diethylhexyladipate and bis (2-ethylhexyl) phthalate, with a total concentration of 1215.77 μg^{-1}. Among them, the concentration of 2, 6-diisopropyl phenyl isocyanate (CAS: 28178-42-9, chemical formula: $C_{13}H_{17}NO$) was the highest, up to 557.27 $\mu g\ g^{-1}$, which was a highly toxic organic compound and was commonly used in the synthesis of medicines, pesticides and polymer material.

Table 1. Component analysis of PBAT-BMF extract.

Name	Concentration ($\mu g\ g^{-1}$)	
	CK	T
2,6-Diisopropylphenyl isocyanate	0.00	557.27
2,6-Diisopropylaniline	0.00	216.81
(Z)-9-Octadecenamide	0.00	373.29
Cyclodecane	0.00	21.42
Bis(2-ethylhexyl) adipate	0.00	24.98
Bis(2-ethylhexyl) phthalate	0.00	22.00

3.2. Effects of PBAT-BMF Extract on Seed Germinative Rate, Germinative Energy and Germinative Percentage

The results of the effects of PBAT- BMF extract on germinative rate, germinative energy and germinative percentage for T-treated and CK seeds are shown in Figure 1. The result of Figure 1a demonstrated that the T-treated process had a certain inhibition on the seed germinative rate compared with CK, the germinative time was one day later than CK, and the germinative rate was slower than CK, but both of them reached the maximum germination on the 10th day. As shown in Figure 1b, the germinative percentage and germinative energy of T-treated seeds were 96.50% and 96.50%, respectively, while it was 99.00% and 97.50% for CK, respectively. Furthermore, germinative percentage and germinative energy of T treatment was lower than 2.50% and 1.00% of CK, respectively, which showed that the difference was not significant ($p < 0.05$).

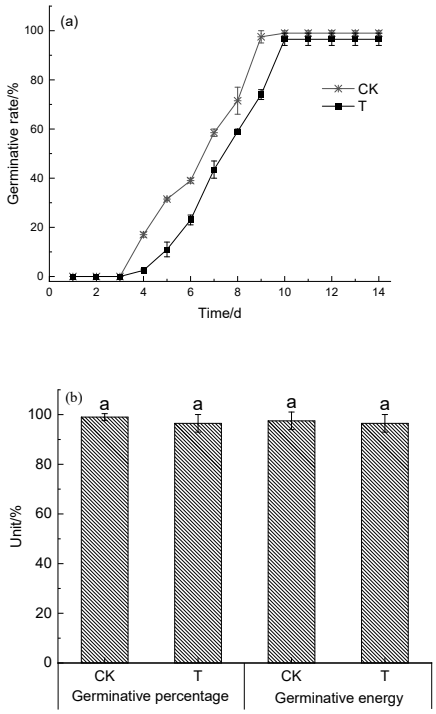


Figure 1. The effect of PBAT-BMF extract on tobacco seed germination, (**a**) germinative rate (**b**) germinative energy and germinative percentage.

3.3. Effects of PBAT-BMF Extract on Root Length and Height of Tobacco Seedlings

The results of the effects of PBAT-BMF extract on root length and height of tobacco seedlings are shown in Figure 2. Results indicated that compared with CK, the root length and height of tobacco seedlings for the T-treated process decreased by 0.24 cm and 0.28 cm, respectively, and the difference was very significant ($p < 0.01$). It demonstrated that the T-treated process had and obvious inhibitory effect on the growth of tobacco seedlings.

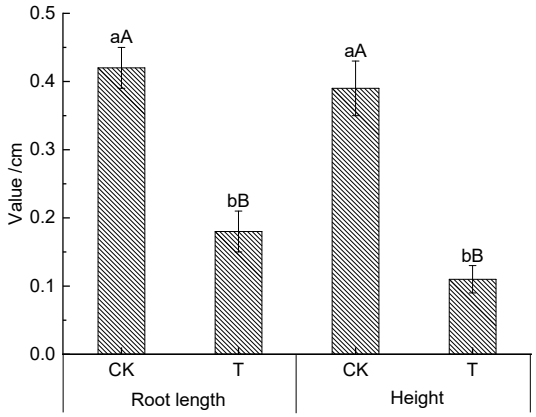
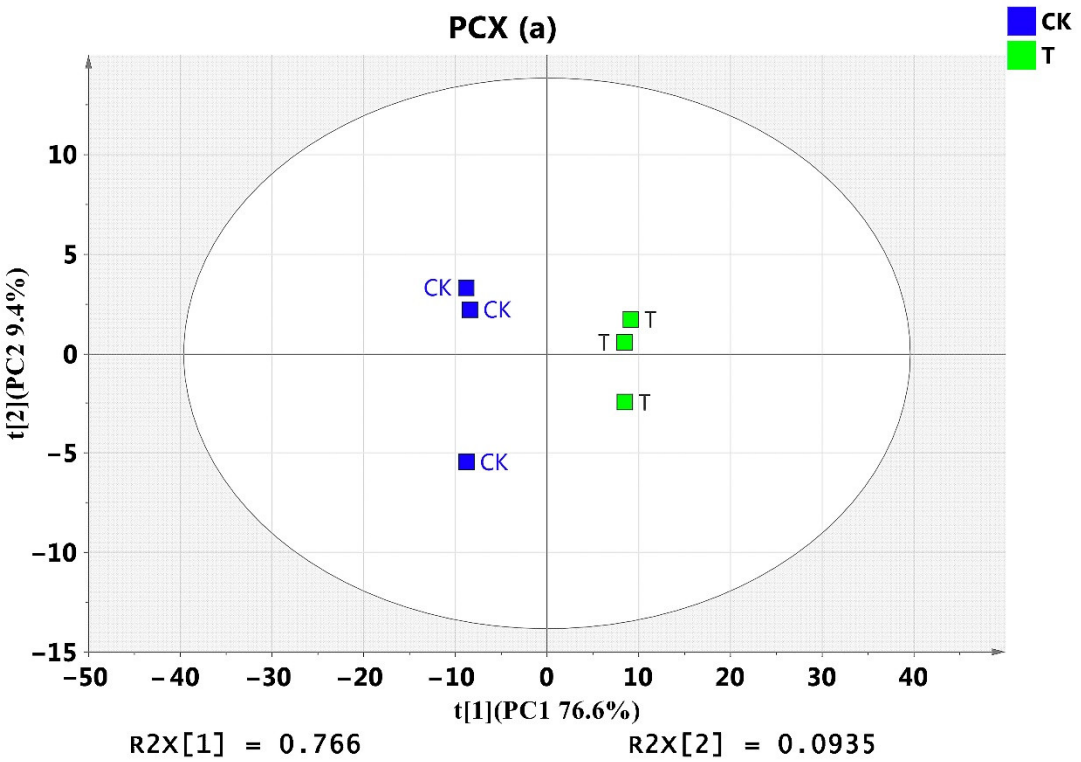


Figure 2. The effects of PBAT-BMF extract on root length and height of tobacco seedlings (A and B were $p < 0.01$ statistical significance, a and b were $p < 0.05$ statistical significance).

3.4. Metabolic Analysis of Tobacco Seedlings

The metabolites of tobacco seedlings treated with T and CK were qualitatively and quantitatively analyzed by the pseudo-target metabonomic method. The results showed

that more than 120 kinds of primary and secondary metabolites were detected, including sugars, organic acids, amino acids, phosphoric acids, biogenic amines, quinic acids, sterols, nucleotides, fat-soluble vitamins and terpenes. In addition, the cumulative explanatory ability R^2X of the model to variable X was 86.4%, and that to Q^2 was 64.1%. The increase of R^2X and Q^2 values indicates that the interpretation ability of the PCA model to the original data was enhanced, and the results showed that the principal component model fitted well. The results of principal component analysis in Figure 3a showed that T treatment and CK were completely separated, indicating that the metabolic pathway of tobacco seedlings changed significantly under stress, resulting in metabolic disorder.



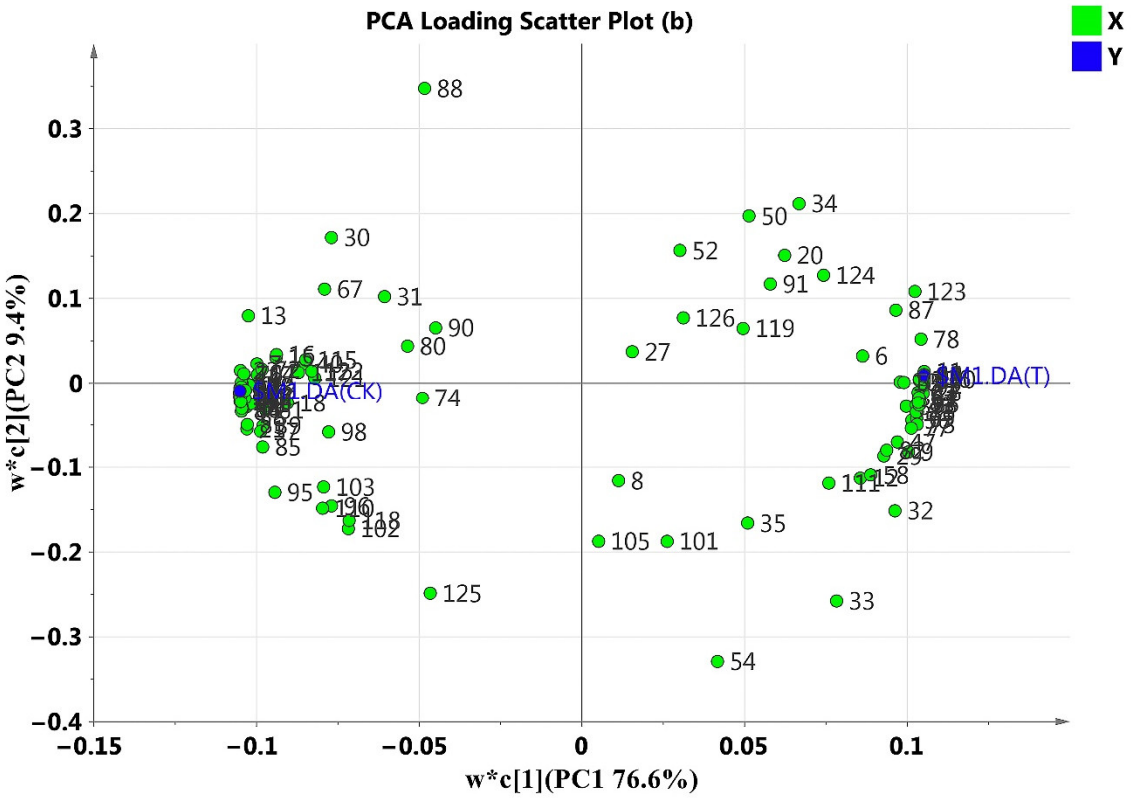


Figure 3. Principal component analysis (**a**) and loading scatter plot (**b**) of metabolites in tobacco seedlings (X (green) and Y (violet) represent the metabolites and sample center point. X (red) represents a potential biomarker).

Moreover, the metabolite loading scatter plot (Figure 3b) based on PCA analysis showed that each circle represents one metabolite, and the results showed that the point where metabolites were closer to T or CK was a potential biomarker. Furthermore, PLS-DA was used to screen potential biomarkers, and 58 metabolites were obtained when *VIP* values were greater than 1.10, which are shown in the heat map (Figure 4). The heat map analysis showed that T treatment caused significant up-regulation of water soluble sugars (glucose, fructose and sucrose) and organic acids in the tricarboxylic acid cycle (pyruvate, 2-oxoglutaric acid, fumaric acid and malonic acid). Biogenic amines (putrescine, phenylethylamine and isopentylamine, etc.) were significantly up-regulated, while alkaloids (nicotine and nornicotine, etc.) were significantly down-regulated. The main amino acids (valine, methionine, pyroglutamic acid, threonine, glutamine, tryptophan, lysine, isoleucine and asparagine) and phosphorylated metabolic pathways (glycerol 3-phosphate, phosphate, diglycerol phosphate and inositol phosphate) were significantly down-regulated.

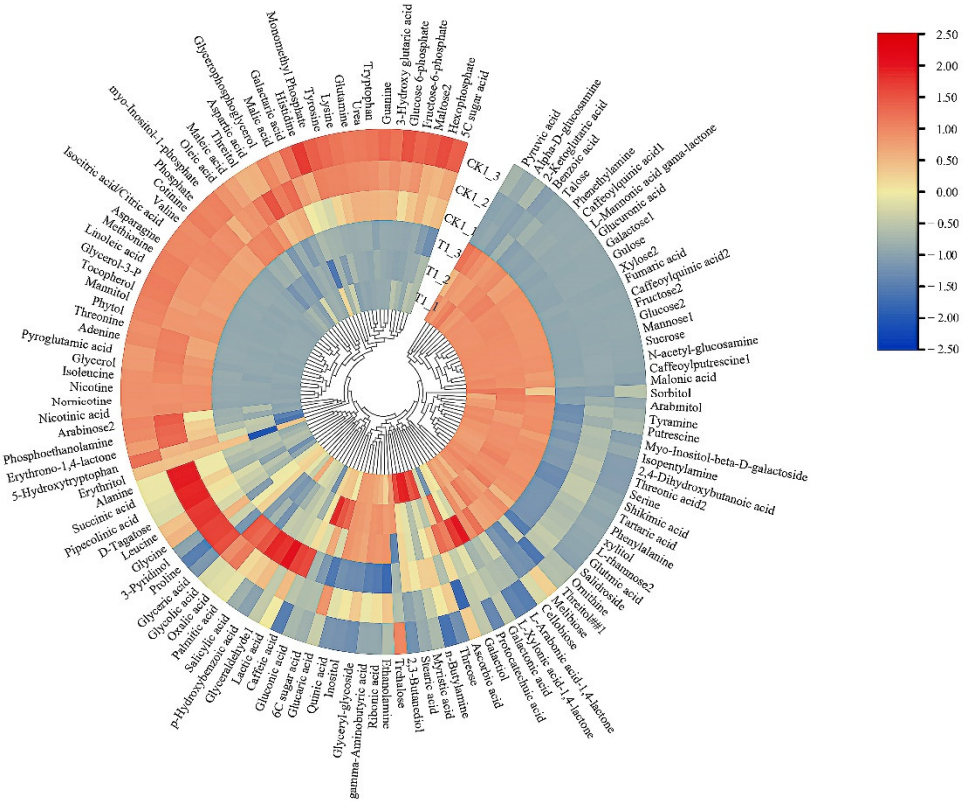


Figure 4. Heat map analysis of metabolites in tobacco seedlings (*VIP* ≥ 1.10).

In addition, the metabolites with *VIP* ≥ 1.10 were tested by T-test significance test, and the final biomarkers were obtained by $p \leq 0.05$. The results showed that a total of 50 biomarkers were obtained under T-treatment, as shown in Table 2. It mainly included sugars (nine species), organic acids (fourteen species), biogenic amines (four species), amino acids (thirteen species), phosphoric acids (three species), nucleotides (two species), alkaloids (three species) and others (two species). Moreover, the metabolic pathways of biomarkers are analyzed and the results of metabolic pathway enrichment are shown in Figure 5, which are mainly concentrated in alanine, aspartic acid and glutamic acid metabolism, arginine biosynthesis, phenylalanine metabolism, aminoacyl tRNA biosynthesis, linoleic acid metabolism, glycine, serine and threonine metabolism, TCA cycle and glyoxylic acid and dicarboxylate metabolism pathways. The results indicated that T treatment mainly interfered with the above metabolic pathways and affected the growth and development of plants.

Table 2. Analysis of differential metabolites in tobacco seedlings.

No.	Metabolites	TConcentration (μg g^{-1})	CK Concentration (μg g^{-1})	Trend	Magnification	VIP Value	Variance Analysis
	Saccharides						
4	Myo-Inositol-β-D-galactoside	6.73	2.17	↑	3.10	1.13	***
11	Sucrose	2942.43	312.00	↑	9.43	1.14	***
12	Glucose2	15,152.93	416.46	↑	36.39	1.14	***
26	Talose	47.83	14.63	↑	3.27	1.14	***
46	Fructose2	14,125.53	3853.90	↑	3.67	1.14	***
62	Xylitol	3.17	1.17	↑	2.71	1.13	***
64	L-rhamnose2	23.40	19.33	↑	1.21	1.13	***
66	Arabinitol	3.40	1.07	↑	3.18	1.13	***
71	Xylose2	39.46	20.63	↑	1.91	1.14	***

	Organic acids						
2	Caffeoylquinic acid	608.80	158.37	↑	3.84	1.14	***
14	Glucuronic acid	11.03	3.73	↑	2.96	1.14	***
22	Linoleic acid	0.23	7.30	↓	0.03	1.14	***
23	Oleic acid	1.77	6.57	↓	0.27	1.14	***
53	Isocitric acid/Citric acid	25.73	87.93	↓	0.29	1.14	***
56	Shikimic acid	19.90	14.17	↑	1.40	1.10	**
78	2-Ketoglutaric acid	1.33	0.37	↑	3.59	1.13	***
81	Threonic acid2	245.40	140.17	↑	1.75	1.12	***
89	Malic acid	264.10	438.83	↓	0.60	1.12	***
93	2,4-Dihydroxybutanoic acid	2.47	1.10	↑	2.25	1.13	***
100	Fumaric acid	46.60	30.33	↑	1.54	1.14	***
104	Maleic acid	20.63	30.97	↓	0.67	1.13	***
107	Nicotinic acid	11.13	32.70	↓	0.34	1.14	***
123	Pyruvic acid	2.37	1.07	↑	2.21	1.11	***
	Biogenic amines						
43	Tyramine	14.37	8.60	↑	1.67	1.12	***
63	Putrescine	1525.23	919.03	↑	1.66	1.12	***
79	Phenethylamine	30.07	4.9	↑	6.14	1.14	***
116	Isopentylamine	11.07	9.17	↑	1.21	1.13	***
	Amino acids						
19	Tryptophan	7.07	11.73	↓	0.60	1.12	***
41	Lysine	7.73	14.37	↓	0.54	1.11	***
55	Ornithine	7.63	5.17	↑	1.48	1.13	**
59	Glutamine	37.94	80.36	↓	0.47	1.12	***
70	Asparagine	3.43	66.07	↓	0.05	1.14	***
73	Phenylalanine	47.63	37.53	↑	1.27	1.12	***
75	Glutmic acid	87.23	64.13	↑	1.36	1.12	***
84	Pyroglutamic acid	222.43	481.87	↓	0.46	1.14	***
86	Methionine	13.23	33.27	↓	0.40	1.14	***
94	Threonine	40.37	59.33	↓	0.68	1.13	***
97	Serine	161.90	98.67	↑	1.64	1.12	***
106	Isoleucine	68.30	104.20	↓	0.66	1.14	***
114	Valine	15.73	25.73	↓	0.61	1.14	***
	Phosphoric acids						
13	myo-Inositol-1-phosphate	0.17	0.57	↓	0.30	1.11	***
60	Glycerol-3-P	1.07	7.80	↓	0.14	1.13	***
108	Phosphate	611.60	1591.37	↓	0.38	1.14	***
	Nucleotides						
28	Guanine	3.00	5.50	↓	0.55	1.11	***
48	Adenine	3.40	8.47	↓	0.40	1.13	***
	Alkaloids						
65	Cotinine	0.37	22.83	↓	0.02	1.13	***
83	Nornicotine	6.43	12.80	↓	0.50	1.14	***
99	Nicotine	843.67	4096.83	↓	0.21	1.14	***
	Others						
25	Phytol	1.13	21.37	↓	0.05	1.14	***
113	Urea	1.93	7.73	↓	0.25	1.12	***

Note: ** and *** denote the significant levels at $p < 0.01$ and 0.001, respectively.

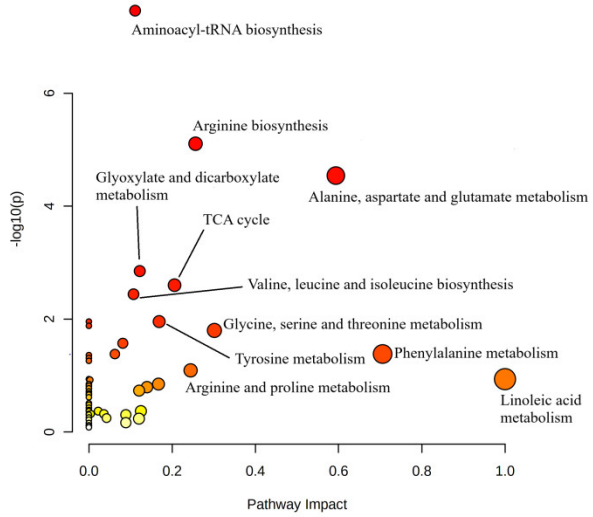


Figure 5. Metabolic pathway enrichment of biomarkers in tobacco seedlings.

4. Discussion

It had been reported that active groups such as the epoxy group, anhydride group and isocyanate were commonly used in PBAT blending modification [27]. In this study, methyl tert-butyl ether was used as extractant to extract six kinds of organic compounds from PBAT-BMF, including 2, 6-diisopropylphenyl isocyanate, 2, 6-diisopropylaniline, (Z)-9-octadecenoic acid amide, cyclodecane, diethylhexyl adipate and di (2-ethylhexyl) phthalate, indicating that the isocyanate active group was used in the synthesis or blending modification of PBAT-BMF. Specifically, 2, 6-diisopropyl phenyl isocyanate, which was often used in the synthesis of medicine, pesticide and polymer materials, had high toxicity and the highest concentration, 557.27 μg g^{-1}, accounting for 45.84% of the accumulated concentration of 1215.77 μg g^{-1} of the six main organic compounds. Although the isocyanate group was a highly reactive one [28], its toxicity and product residues need to be further studied. In order to ensure the safety of biodegradable materials or products, it was suggested that isocyanate groups should be replaced by epoxy groups with higher reaction activity but lower toxicity in the process of material synthesis or blending modification [29].

In addition, germinative energy and percentage of seeds reflected the germinative ability and emergence uniformity [30], indicating the production performance of seeds [31]. The plant root system was an important organ for plants to obtain nutrition and water, which was closely related to plant stress resistance and yield [32], and played an important role in plant growth [33]. Through the germination experiment of PBAT-BMF extract containing 2, 6-diisopropylphenyl isocyanate and 2, 6-diisopropylaniline, it was found that the PBAT-BMF extract had a certain inhibition on seed germination, such as delayed seed germination time, slower germination rate, lower germinative energy and percentage, and weaker root length and height growth of seedlings. The extract of PBAT-BMF also had a stress effect on the metabolism of tobacco seedlings, which was mainly characterized by the up-regulation of water-soluble sugars and organic acids in the tricarboxylic acid cycle. This may be due to the alienation of polysaccharides such as starch in tobacco seedlings, which degraded to produce water-soluble sugars and provided more energy for tobacco seedlings. The significant up-regulation of organic acids in the tricarboxylic acid cycle further proved the improvement of energy metabolism. Biogenic amines were significantly up-regulated and alkaloids were significantly down-regulated, indicating that alkaloid synthesis was blocked under stress, mainly accumulating biogenic amine metabolites in the synthetic pathway.

Previous studies had shown that biogenic amines were closely related to plant stress resistance, which could respond to plant stress resistance by scavenging active free

radicals, regulating the cell membrane ion channels and the calcium ion balance [34]. The main amino acids and phosphorylated metabolic pathways were significantly down-regulated, indicating that the PBAT-BMF extract might affect the absorption of nitrogen and phosphorus by tobacco seedlings. Glutamine and asparagine were the main N-rich amino acids in leaves and participate in the fixation of inorganic nitrogen. The reduction of these two amino acids proved the interference to the process of nitrogen metabolism. Enrichment analysis further proved that the stress of PBAT-BMF extract on tobacco seedlings had a comprehensive impact on the absorption and metabolism of carbon, nitrogen and phosphorus.

The composition of PBAT biodegradable plastic film was complex, so it was particularly important to evaluate the potential ecological risk of its environmental friendliness or tolerance [35]. In this study, the seedling germination experiment of the PBAT-BMF extract was carried out, and it was revealed that the extract had some negative effects on the germinative rate, energy, percentage, growth and metabolites of tobacco seeds. But which or what kind of organic compound has the inhibitory effect? What is the relevant mechanism? In depth research will be carried out in the future.

5. Conclusions

In this study, methyl tert-butyl ether was used as an extractant to extract 2, 6-diisopropylphenyl isocyanate, 2, 6-diisopropylaniline and (Z)-9-octadecenoic acid amide from PBAT-BMF. The extract had stress effects on the growth of tobacco seeds, as well as on the metabolism of tobacco seedlings, affecting the carbon and nitrogen metabolism processes such as the tricarboxylic acid cycle and alkaloid synthesis. This study provided a theoretical basis for assessing the ecological safety in the condition of long-term biodegradable mulch film.

Author Contributions: Conceptualization, W.G.; methodology, K.C.; software, Y.L.; validation, W.G., and K.C.; formal analysis, H.L.; in-vestigation, D.P.; data curation, W.P.; writing—original draft preparation, Z.L. and D.P.; writing—review and editing, D.P. and J.F.; visualization, Z.L.; supervision, W.G.; project administration, W.G.; funding acquisition, W.G., K.C., W.P. and J.F. All authors have read and agreed to the published version of the manuscript.

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