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Glucosinolates Detoxification by Solid State Fermentation as Fungi "Biological" and Copper Sulfate Solution "Chemical" Methods

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Abstract

The seeds of Brassicaceae or Cruciferae family contain secondary plant products with various biological effects, including beneficial and harmful ones. These products contain glucosinolates (Gls). They have many types, but the bitter taste type and the anti-nutritive effect are thioglucosides, which contain a sulfate group on the aromatic ring. The seeds of the watercress plant, which were used in the current study in order to try to get rid of the impact of those substances, by cracking by the enzyme myrosinase and is able to crack and decompose Glycoside bond, a water enzyme, and Using six fungal strains: *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Trichoderma longibrachiatum*, *Penicillium digitatum*, and *Fusarium oxysporum* in addition to one chemical treatment using hydrothermal copper sulphate ($CuSO_4$), and comparing the previous seven fungal and chemical biological parameters with a control treatment in which the parasite was treated The seeds of watercress are the same as the pre-treatment in the preparation of the solid fermentation environment but without fertilization of the environment in any fungal chain and the control treatment was incubated in the same conditions as the six fungal treatments. Because the seeds of watercress seeds are rich in antioxidants and anti-cancer, These materials are in all experimental and knowledgeable transactions The following tests were performed: Total polyphenols, Total flavonoids, Reducing power, $ABTS^+$ action radical, DPPH radical, and Hydrogen peroxide scavenging activity, in addition to analysis The results of the various chemical treatments from the normal chemical analysis of protein, fiber, ash, fat and carbohydrates as well as the major mineral elements, to determine the effect of each treatment on the seedling of watercress seeds (*Eruca sativa* meal). The biological treatment included natural thermal treatment by sterilization of the plant material and biological fungal treatment by pollination and incubation and then the secretion of the water enzyme Capable of cracking glucosinolate for other substances. The results of this study showed that there are three biological factors which are T2 (*Macrophomina phaseolina*), T4 (*Trichoderma longibrachiatum*), and T6 (*Penicillium digitatum*) achieved the highest ratios that exceed the control treatment (Non-biologically and chemically treated), in the measurements of total polyphenols and total flavonoids content, Reducing power, ($ABTS^+$) action radical, (DPPH) radical, and Hydrogen peroxide (H_2O_2) scavenging activity of rocket meal seed (RMS) in different treatments.

Keywords

Solid Fermentation, Glucosinolates Detoxification, Copper Sulfate Solution, Antifungal Activity, Isothiocyanates, Antioxidant Activity, Chemical Composition, Watercress Plant (Rocket Seed Meal "RSM")

1. Introduction

1.1. Fungal Strains Used in the Solid Fermentation and Its Role in Removing Toxic Glucosinolates (Glucosinolates Detoxification)

Using ITCs individually, multiple responses to the inhibition of fungal pathogens have emerged and The fungus is usually determined by the presence of the toxic compound as an initial point of the fungal growth. The toxic compounds produced by the fungus are used to describe the fungi. The secondary product of its growth [1]. Reports with *Fusarium oxysporum* by Smolinska *et al* [2] The constant reaction of toxic responses by toxic fungi was to determine the concentration of fungi in the medium and this was presented to Inyang *et al.* [3], By using laboratory methods " in vitro" that ITCs can be prevented conidial germination and mycelia growth of pathogenic fungi of *Metarhizium anisopliae*. About the state of being exposed to contact with something to allyl and benzyl-ITC have been identify the presence Fungi toxic belongings on the mycelial growth of *Alternaria spp*. [4, 5].

As part of their protection system in *Brassicas*, The ITCs are produced, these biological combinations with potent antifungal motion. The application of chemical composites for controlling fungal contaminations in agricultural products. the fungal molecular mechanisms answering to the toxic effect of ITCs.

A sophisticated plant that is grown as food, particularly a grain, fruit, or vegetable play an imperative role in vital interactions in nature [6]. Antimicrobial phytoanticipins and phytoalexins, respectively, plant defenses depend on constitutive and the pickets induced many workers to stay away production [7, 8]. *Arabidopsis thaliana* and *Phytoalexin camalexin* for the model plant [9] it is one of the important elements of resistance to fungal pathogens nerve [10]. Fungal detoxification mechanisms are exposed to antimicrobial activity of camalexin [11], with the accumulation of camalexin in the growth environment of Botrytis cinerea, resistance cannot even be half the toxicity of the control environment [12]. Glucosinolates (GSLs) are referred to as glucosides of mustard oil, and have an effect of phytoanticipins and are of economic importance to Brassicaceae [13]. The degree of biological activity depends on the degree of secretion of the myrosinase enzyme that stimulates its hydrolysis [14], A compound that inhibits the growth of many pathogens in the laboratory is a 4-methylsulfinylbutyl isothiocyanate compound, and abbreviated to him (4MSOB-ITC) [15].

1.1.1. Glucosinolates Detoxification by *Sclerotinia sclerotiorum*

Secondary plant metabolic products work with a variety of favorable and tending to cause harm organizations to facilitate interaction, but the degree of substantial contribution of relating to or deriving from the metabolism of a living organism to fungal pathogens is not known specifically, from glucosinolates derived from the aliphatic ITCs compound, which has toxicity against diseases caused by S. sclerotiorum, Its system of lawsuit on the temple of the quarter chain, as well as the genes associated with the secondary metabolites of both camalexin and glucosinolate [16]. Scheme representation of enzymatic hydrolysis of glucosinolate (Figure 1). It was absorbed that in Arabidopsis thaliana leaves used euphemistically with pathogen *S. sclerotorum*. Unlike *S. scleroturum*, the cytotoxicity ascomycet of Botrytis cinerea is not closely specified to induce gene-related aliphatic bio-glucosinols in the pathogenic pathogens. The incomplete mutant lines in camalexin, endol, or aliphatic glucosinolate were highly apt to *S. scleroturum*, which has a overseer defect resulting in a decrease in the production of long-chain aliphatic GLs. The antimicrobial bounce of glucosinolate-derived GLs was based on elongation and modulation of side-chain, with 8-methyl-sulfhenylactyl isothiocyanate (8-MSITCS) being the most toxic of *S. scleroturum*. This is important for relatives of microbial killers with host plants and metabolic engineering of pathogenic defenses in former crusader plants that produce aliphatic GLs [17].

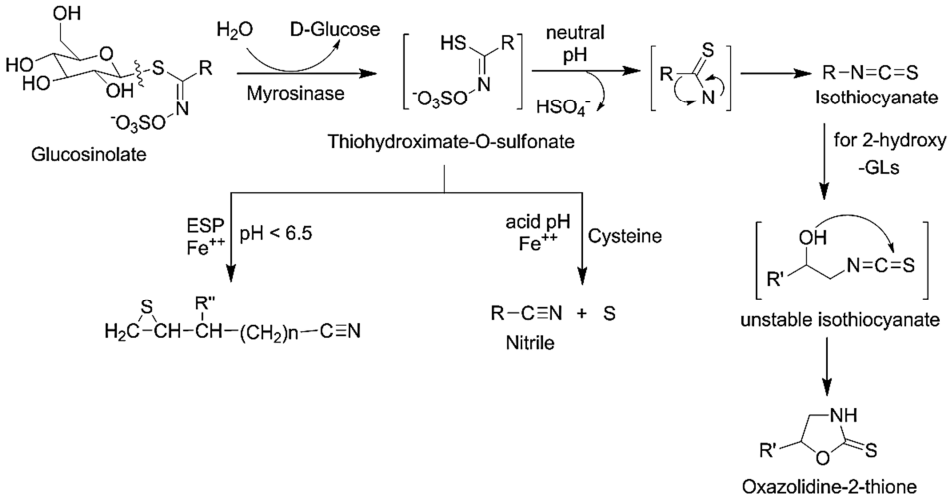

Figure 1. *Diagram representation of enzymatic hydrolysis of glucosinolate.*

The rejoinder of *Sclerotinia sclerotiorum* (Figure 2), Agent of the trunk stem of rapeseed oilseeds (*Brassica napus*), the myrosinase gluosinolate system was a toxic volatile substance manufactured. The fungal plugs of the forbidden tablets of the oil rape seed varieties and; white and black mustard two united species were exposed. The growth of non-protected colonies was introverted by more than 87% of the controls. In spite of the inhibition of open fungal colonies, fungi are increased in infected tissues. Repeated exposure to the mustard powder (containing both glucosinols and myosinase) or artificial islets (ITC) occurred in the growth of inhibition of reduction after two to three days from former levels up to 80% to insignificant levels, put forward for consideration that *S. sclerotiorum*. Solid has the skill to fit to volatiles through corruption advance. This adaptation was investigated by investigating the induction of S-transgenase glutathione genes such as the specific genes of the *S. sclerotiorum* genome. Three genes appeared on their up-regulated *S. sclerotiorum* exposure to mustard powder. The fourth gene appears to be descending down. In the supplement, catalytic activity of S-transferase glutathione was doubled in fossil extracts 2 days (Forty eight hours) next insinuation to volatile mustard powder. This adjustment can allow *S. sclerotiorum* to parasitic matters of the *Brassica* kinds notwithstanding the output of toxic metabolism [18].

Sclerotinia sclerotiorum sur une tige de coco de Paimpol (*Phaseolus vulgaris*) (Ploulec'h, Côtes d'Armor, Bretagne, France).

Sclerotinia sclerotiorum on bush bean

Figure 2. *Sclerotinia sclerotiorum (From Wikipedia, the free encyclopedia).*

Indole-3-acetaldexime is as follows: (1)- Medium vital importance from other secondary metabolites of Cruciferae. (2)- Indole-3-acetaldexime metabolism to endol-3-acetic acid by endol-3-acetonitrile by a fungus that causes paramount plant diseases in syphilis, Leptosphaeria maculans causing black disease, root rot disease causing serotonin Rhizoctonia solani, while the causative factor of rot Sclerotinia sclerotiorum stem. (3)- The antifungal activity of endol 3-acetaldexime, metabolites, synthesis and the alteration of a substance of 4- hydroxyphenyl-acetaldoxime by plant pathogens themselves and bug fungal pathogens, (4)- can be quantified [19].

Host cell death and widespread tissue damage are caused by necrotic diseases [20]. There is a wide range of host Sclerotinia sclerotiorum with the aggressive Necrotrovic nurse [21]. Facilitate the death of host cells and modify the types of reactive oxygen that occurs by excretion of oxalic acid from this ascomycete [22, 23]. Hydrolysis of glucosinolate, known as the myrosinase hydrolysis system, occurs to produce selected chemical structures from ITCs by secreting dead host cells of the enzyme myrosinase (Figure 3) and GSLs, Toxic products will be present in their midst [14]. ITCs are created by default in the midst of these toxic products, and there must be specific proteins that intermingle with the myrosinase to adjust their movement to produce nitriles and epithiontrile [14, 24, 25].

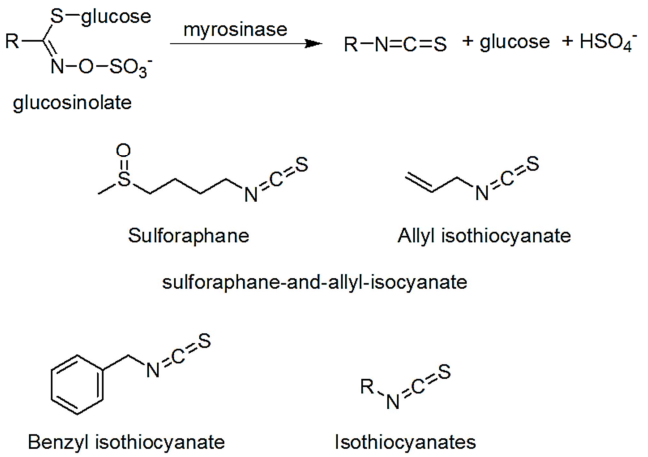

Figure 3. *Myrosinase-catalyzed glucosinolate hydrolysis and chemical structures of selected ITCs.*

Recently, neurotropic patho-system was the collaboration between *S. sclerotiorum* and *A. thaliana* [26, 27]. Alongside

S. sclerotiorum was a manifold network of hormonal and pointing trails normalizes defense responses [28]. This shield network includes of positive regulators of a jasmonic acid, ethylene, abscisic acid (ABA), and nitric oxide [27, 28]. The tough susceptibility of the jasmonate receptor mutant coil to S. sclerotiorum septicity was the aim the present study [27]. The plant defensin PDF1.2 after S. sclerotiorum encounter was gridlocked in this altered, signifying interposition with exclusive jasmonate-dependent sentinel rejoinders.

This report a transcriptase analysis of A. thaliana polluted with S. sclerotiorum, which evaluation of mutants with faults in GSL biosynthesis and camalexin. Unambiguously, the pad3 prejudiced malfunctioning in the latter step of camalexin biosynthesis [9], In the first fanatical step of camalexin and indole GSL biosynthesis there is a double blemish mutant to the cyp79b2 / cyp79b3 [29], and explicit aliphatic GSLs were used mutants in three coupled R2R3 MYB transcription influences [30-32] for the tenacity of trying the hero of GSLs and camalexin in resistance beside S. sclerotiorum.

Canola (*Brassica napus*) is largest oilseed crop in the world after soya bean, propounding thirteen percent of the world's supply [33]. Over the past two decades, this crop has accepted through peanuts, sunflower, and cotton seeds in world production. Shoot rot caused by *Sclerotinia sclerotiorum*isa is a major disease of canola in the main areas of crop construction [34]. Australia's canola agriculture areas have become one of the most kind health complications [35]. *Sclerotinia sclerotiorum*is have the capacity to attack and disease more than four hundred species of plants in Seventy-five different families [36].

Canola and its relatives, black and white mustard of *Brassicaceae* oilseeds, produce by-products termed glucosinolates (GSLs). It is hydrolyzed by the enzyme myrosinase, which is bottomless due to commotion of cell honesty [37]. This outcomes from the putrefaction feedback enzymatic a wide range of special buses with bio-pesticides ITCs properties, with the sway on living organisms such as insects, fungi, and plants [38]. The toxic effects of ITCs have been corroborated in the laboratory *S. sclerotiorum* [39, 40]. Native GSLs unaided did not have toxic belongings on the fungus.

There is buoyant character for these toxic products from GSL predominantly in the resistance of plants comprising GSL hydrolysis alongside fungal pathogens. The GSL of *Arabidopsis thaliana* changed its resistance to bacterial and fungal pathogens [41]. Some pathogens and hassles of the *Brassica* species have been identified from ITCs. Sexton *et al*. [42], announce no relationship between the near of allyl-GSL and black leg struggle in stems cotyledons of *B. juncea*, *in vitro* poisonousness of allyl-ITC to the pathogen. That powerful isolates of *L. maculans* escape the toxic effects/might detoxify of hydrolysis products from this GSL. Furthermore, *Alternaria brassicicola*, a fungal pathogen of *brassicas*, mainly canola, demonstrated tolerance to benzyl ITC [4, 5]. That *S. sclerotiorum*, may also be able to discharge the toxicity of the system of GSL-myrosinase, although it has a wide range of pathogens towards *brassicas* [43].

The capacity to detoxify chemical defense compounds may be relentless again. Pedras *et al*. [44] have offered that purify phytoalexins from *Brassica* species an inducible glycosyl transferase qualified *S. Sclerotiorum*. Cramer and Lawrence [45] revealed that a cyanide hydratase-encoding (CyhAB) gene, was up-regulated through the collaboration of *A. thaliana* with *A. brassicicola*, and that this enzyme may be for the detoxification of GSL-break down products. Freshly, Sellam *et al*. [4, 5] have recognized that scrambling a glutathione S-transferase (GST), was the gene AbGst1, in the same fungus on coverage to ITC. This enzyme exhibited high transferase action with ITCs. GST is thus a candidate for ITC detoxification through host infection. The expression of numerous putative GST-encoding genes was encouraged by a half an hour exposure of *A. brassicicola* conidia to allyl ITC and benzyl ITC. Allyl ITC increased illustration from twenty-five to thirty times paralleled with controls [46].

1.1.2. Glucosinolates Detoxification by *Macrophomina phaseolina*

Macrophomina phaseolina, is the pathogenic agent of coal-rot fungus borne by soil and seeds. It causes diseases of more than 500 different types of crops, and it causes heavy economic losses both in legume crops such as cowpea, beans, peanuts, soybeans or Narcotic crops (grassy) such as corn, sorghum and cotton. [47]. The fungus spread throughout the world, more than their economic influence in the subtropical states, and strictly impact in semi-arid countries [48, 49]. When the cell is interjected when the formerly separated enzymes and substrates interact, only the packet compounds are released [50]. The celery "*Apium graveolens*", the lactoca *"Lactuca sativa*", contains some aromatic complexes for sugars as well as glycosides, or the cabbage *"Brassica oleracea"*, and the radish *"Raphanus sativus*" contains glucosinolates. This correlation can be ended by enzyme action or heat during catering. also, other aromatic compounds can pause down such fats, amino acids, lignin, and dyes [51].

1.1.3. Glucosinolates Detoxification by *Alternaria alternate*

Studies show that fungus *Alternaria brassicicola* comprises three genes that comedy a very significant role in oxidative trauma. These genes are: cytochrome P450, thioredoxins, and oxidoreductases. These genes play an authoritative role within the plant cell to destroy your ITCs, namely three compounds, glutathione-*S*-transferases, and ABC transporters, are three genes that disregard three toxic compounds. A pathogen dedicated in *Brassicas* is the fungus *A. brassiciccola*, while a generalist pathogen is *Alternaria alternata*. Though, a gene drill an ABC transporter in *A. alternata* it was also carried the induction of. That the fungi rejoinder apparatus to ITCs consists in the formation of an isothiocyanate-glutathione conjugate which is obscured by an ABC transporter. That the exploitation of ABC transporter

inhibitors with ITCs in unification, could elude the fungal endurance and for human health as well as innocent treatment for the atmosphere [52].

Troncoso-Rojas *et al.* [53] have stated that the ITCs mode of action is not entirely understood, ITCs in *Alternaria alternata* is destroyed by low concentrations of 2-propenyl-isothiocyanate [39]. For example, *A. brassicicola*, and *A. brassicas* are species of *Alternaria* genus which operates ITCs a defense mechanism, able to pollute species of *Brassica* spp. [54]. Benzyl-ITC yields are the hydrolyzed glucosinolate of *Alternaria alternata* [39], The percentages of the following compounds came to some (1) allyl: (3.5) benzyl: (5.3) 2-phenylethyl: (9.6) phenyl ITCs [55] and add them to the fifth boat "3-methylsulfinylpropyl ITC" [39].

1.1.4. Glucosinolates Detoxification by *Trichoderma longibrachiatum*

Trichoderma species use the competition for nutrients and/or space, the antibiosis and/or the mycoparasitism to control different phytopathogens. In accumulation, new mechanisms have been found in some species and strains of *Trichoderma*, for instance: the inactivation of the enzymes of the pathogen, the detoxification of antibiotic substances or antimicrobial compounds produced and freed by the fungus host and/or by the micro flora of the soil, or in an indirect stimulation of justification mechanisms of the host plant [56, 57].

1.1.5. Glucosinolates Detoxification by *Penicillium digitatum*

The presence of tocopherols in mustard seed sponsors to its ledge life. Mustard powder is about 28-36% crude protein [58]. Mustard is famous for its loud taste, an essential element of bandages and pulp. Mustard oil converts without the growth of some yeast, molds, and bacteria. There are about 26 compounds that are natural preservatives. Allyl isothiocyanate (AITC) vapor significantly condensed the growth of the causal agent blue mold on pears, *Penicillium expansum* [59].

Allyl isothiocyanate (AITC) vapor beside wild-type of the fungicidal stuff and *Penicillium expansion* has been demonstrated of thiabendazole-resistant strains [59, 60]. Biocidal movement against a wide assortment of organisms for sample insects, plants, fungi and bacteria of GLS hydrolysis products [60, 61]; As well as some health benefits for humans. ITCs are the major inhibitors of microbial activity midst the most potent products. sometimes, used as an antibiotic to luxury infections of the sentient and urinary tract by Benzyl ITC [62]. ITCs are well celebrated bacteriostatic, bactericidal, and fungicidal effects [38]. Thiabendazole-resistant strains of *Penicillium expansum* has been founded and The fungicidal belongings of Allyl isothiocyanate haze against wild variety [60].

1.1.6. Glucosinolates Detoxification by *Fusarium oxysporum*

In *vitro*, tested Eleven GSLs and their enzymatic hydrolysis yields against *Fusarium culmorum* [39]. Supplementary, deficient in producing the 4MSOB-ITC precursor of the gsm1 gene mutant of A. thaliana is vulnerable to extreme chlorosis upon adulteration by Fusarium oxysporum. for the gsm1 gene has not been cloned and the mutant is also exhausted in another antimicrobial compound, the isothiocyanate (ITC) is unclear the distrustful character. Indole GSL catabolic passageway was recently shown to stimulate native immunity, motivating in protection against fungal permeation [63, 64]. Motionless, GSL-stemmed antimicrobial defenses remains encounter of the mechanical basis. Allyl-ITCs are things of *Fusarium oxysporum* that effect on glucosinolate hydrolysis [65], Examples are alkenyl aliphatic ITCs: methyl-ITC, propenyl-ITC, butenyl-ITC, pentenyl-ITC, propenyl-ITC, ethyl-ITC, benzyl-ITC, and phenyl ethyl-ITC [2].

When *Fusarium oxysporum* was an initiate to be significantly more and/or extra?!; When they practiced the resistance of an Arabidopsis MAM1 (methyl thioalkymalate synthases, MAM) mutant which after damage exhibits a lower amount of 4-methyl-sulphinyl butyl isothiocyanate, *F. oxysporum* was educated to be significantly more antagonistic on gsm1-1 (Code and gene number) gene mutant, a mutant flawed in aliphatic GLs than on wild-type plants, that GLS-derived ITCs might play a role in the fortification of Arabidopsis against evident pathogens only *F. oxysporum* was announced to be significantly extra destructive than on wild-type plants [15].

1.2. Action with H_2O and Copper Solutions

The incorporation of copper sulfate preserved RSM in the diet 80 g Kg^{-1} of broilers and 160 g Kg^{-1} (any quantifiable paleness) of pigs improved growth, thyroid meaning, iodine grade, serum Zn relaxed and alkaline phosphatase action. The helpfulness of $CuSO_4.5H_2O$ action on GLs, ITC and VOT content was also related but how the copper sulfate treatment eliminated GLs and/or ITC and VOT subjects of the meal is debatable. Nonetheless reduced 3-indolylmethlglucosinolates from *Brassica napus* leaves by the mean of metal ion treatment was patrolled [66]. Conceivably copper sulfate dealing removed glucosinolates degradation reactions or rearrangement of metabolites took place running to ITCs and/or nitriles production, which is volatile in nature. Another possibility seems from the reorganization reactions the production of amines such as allyl-amine or thio-urea [67].

2. Materials and Methods

The succeeding experiments were passed out at Chemistry Department, and Plant Pathology Department, Faculty of Agriculture, Mansoura University, Aljumhuria Street, Mansoura City, Egypt.

2.1. Solid State Fermentation

Solid fermentation was done on the experimental material (Rocket seed meal "RSM") considered using the

strain *Aspergillus oryzae*, It has been attained from the Department of Agricultural Microbiology, Faculty of Agriculture, Damietta University, Damietta, Egypt. Solid state fermentation of rapeseed meal or rocket meal (sterilized at 121°C, 15 min) "(°C) Degrees Celsius" using *Aspergillus* sp. and *Rhizopus oligosporus* under solid state fermentation (meal: water ratio 1:3, at 25°C under aerobic condition, 10 days) disabled myrosinase, abridged total Gls by 431mol/mmol and *thiooxalidone* by 340mg g−1 [68]. The overall mortification of Gls happened after 2.5-4 days (60–96 h) fermentation at Twenty-five degrees Celsius (25°C) [69]. However, in the current study, the fermentation duration was extended for 10 days to ensure the best results of Gls breakdown.

2.2. Fungal Isolates

The isolates of *Sclerotinia sclerotiorum* (The fungus was isolated from the fruits of islands affected by sclerotin mold), *Macrophomina phaseolina* (AUMC 10204), *Alternaria alternate* (AUMC 10301), *Trichoderma longibrachiatum* (AUMC 5990), *Penicillium digitatum* (It was isolated from the fruits of an orange fungus infected with green mold), and *Fusarium oxysporum* (f. sp. *Lycopersici* AUMC 9704) were attained from Assiut University Mycological Centre (AUMC) and the Plant Pathology Institute, Agricultural Research Center (ARC), Egypt. The fungi, isolated from a potato tuber, were maintained on potato dextrose agar (PDA) slants. The agar slants were stored at Four degrees Celsius (4°C) and served as stock cultures.

2.3. Copper Sulfate Treatment

Among various RSM action protocols (soaking meal with H_2O, Cu, Fe, Ni, Zn sulfate or copper sulfate solutions), only copper sulfate treatment was found effective, which inactivated ITCs, and 5-vinyl-2-oxazolidinethione (VOT) of the meals. Discussing RSM with copper sulfate [0.5 Kg RSM soaked in one liter copper sulfate solution, 3.125 gm $CuSO_4.5H_2O$ dissolved one liter H_2O and dried at Sixty degrees Celsius (60°C)] was effective in reducing Total Gls by 900 µmol/mmol [70, 71].

2.4. Total Polyphenols and Total Flavonoids Content of *E. sativa* Meal

Total phenolic contents of the air dried meal were determined by using method according to El-Fadaly et al. [72, 73], also Lin and Tang [74]. Total flavonoids content of air-dried meal were determined colorimetrically using aluminum chloride as described by Chang *et al.* [75].

2.5. Reducing Power of Plant Treatments

Reducing power of plant treatments was determined according to the method of El-Fadaly *et al.* [72, 73], and Oyaizu [76].

2.6. Antioxidant Capacity of Treatments Determined by ($ABTS^+$) Action Radical

ABTS (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of El-Fadaly *et al.* [72, 73], and Re *et al.* [77].

2.7. Antioxidant Capacity of Treatments Determined by (DPPH) Action Radical

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of extracts at different concentrations were measured from bleaching of the purple color of (2.2-diphenyl -1-picryl hydrazyl) was based on the method of El-Fadaly *et al.* [72, 73], and Pratap *et al.* [78].

2.8. Hydrogen Peroxide Scavenging Activity

H_2O_2 Scavenging potential of plant extracts was analyzed by reported method [79] with slight modifications.

3. Results and Discussions

Glucosinolates are existing in the seed and other parts of oilseed rape, befall as glucosides throughout the *Brassicaceae*, These the hydrolysis profits of GLs include nitriles, ITCs, oxazolidinediones, and thiocyanates, which can be unpleasant and toxic to single stomach (non-ruminant and/or semi-ruminant) animals, Such as poultry, pigs, and rabbit, Because they do not have a multifaceted containing microbiological digestion such as ruminants. the presence of GLs in rape meal has restricted its use in animal feed, demanding the enlargement of the so-called 'double-low' lines. These produce seed low in erucic acid and Gls. A decreased content of GLs in oilseed rape tissues may have negative significances for pest and disease occurrence on the crop. The hydrolyzed products of GLs are fungi-toxicity to rapeseed pathogens *in vitro* [80] and that their existence subsidizes to endurance to a range of oilseed rape pathogens [81, 82]. Following immunization with *Alternaria brassicae,* Doughty *et al*. [83] have spotted the response varied on leaf age and cultivar and found that Gls contents increased markedly, but the changes in the GLS content of leaves in cultivars Bienvenu (single low) and Cobra (double low). Gls ensued more in the sixth leaf of Bienvenu. When *B. rapa* seedlings are vaccinated with *A. brassicae*, 3-butenyl and 4-pentenyl ITCs are reduced, together with dimethyl disulfide, 4-oxoisophoron, and a number of sesquiterpenes. The release of ITCs is an evidence for the catabolism of Gls through infection, which is a prerequisite for their involvement in defiance [83]. The (DE) values were determined by the method reported by Fekete and Gippert [84], Chemical composition of meal coefficient natural, biological and chemical transactions, to measure the change in the composition after these various transactions (Table 1), While displaying Table 2, Analysis of metallic elements in the samples treated with different transactions.

Table 1. *Chemical composition of meal coefficient natural, biological and chemical transactions, to measure the change in the composition after these various transactions.*

NO.	Moisture%	Ash%	Crude fiber%	Crude protein%	Crude lipids%	Total carbohydrate%	Soluble carbohydrate%	DE*
1	8.42	6.8	18.97	42.97	1.8	29.46	10.49	2652.66
2	9.63	7.0	16.58	43.45	2.0	30.97	14.39	2701.69
3	8.75	7.2	18.41	43.07	1.9	29.42	11.01	2613.15
4	10.09	6.9	15.33	43.74	1.7	32.33	17.0	2756.88
5	9.94	7.1	15.91	43.58	1.7	31.71	15.8	2709.09
6	9.33	7.1	17.20	43.34	2.1	30.26	13.06	2667.04
7	9.04	6.9	17.85	43.18	2.0	30.07	12.22	2674.73
8	8.13	7.0	19.62	43.63	1.8	27.95	8.33	2602.59

No. Treatments: (1) Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days), (2) Control + *Fusarium oxysporum*; (3) Control + *Macrophomina phaseolina*; (4) Control + *Sclerotinia sclerotiorum*; (5) Control + *Trichoderma longibrachiatum*; (6) Control + *Alternaria alternate*; (7) Control + *Penicillium digitatum* and (8) $CuSO_4$.

DE* (Kcal/Kg DM) = 4253 – 32.6 (CF%) – 144.4 (Ash%).

% Total carbohydrate = 100 – (Ash + Protein + Lipids + Fiber).

% Soluble carbohydrate =% Total carbohydrate -% Fiber.

Table 2. *Analysis of metallic elements in the samples treated with different transactions.*

NO.	mg/100 gm						mg/1 Kg			
	P	K	S	Na	Ca	Mg	Zn	Cu	Fe	Mn
1	84.2	304.5	348	27.6	405.3	95.8	3.81	2.07	8.29	3.66
2	72.6	293.6	245	19.9	365.6	105.9	3.35	1.42	7.38	4.42
3	81.9	301.7	319	25.3	394.8	98.2	3.96	1.91	6.49	3.85
4	66.7	288.1	198	16.7	347.2	112.1	2.93	1.05	8.02	4.85
5	69.5	290.7	223	18.2	356.3	108.3	3.48	1.17	7.71	4.60
6	75.7	296.4	271	21.4	374.9	103.5	3.09	1.58	7.09	4.21
7	78.8	298.5	296	22.8	385.1	100.8	2.93	1.75	6.80	4.03
8	87.4	307.6	371	28.9	416.8	114.7	3.74	2.19	8.57	4.95

No. Treatments: (1) Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days), (2) Control + *Fusarium oxysporum*; (3) Control + *Macrophomina phaseolina*; (4) Control + *Sclerotinia sclerotiorum*; (5) Control + *Trichoderma longibrachiatum*; (6) Control + *Alternaria alternate*; (7) Control + *Penicillium digitatum* and (8) $CuSO_4$.

Cooked Watercress (Heat Treatment Wetlands)

Cooking vegetable destroys myrosinase enzyme, it is important to examine whether dietary GLs are really rehabilitated to ITCs after consuming cooked vegetables. A urinary symbol, based on a cyclo-condensation manufactured goods designed by the consequence of ITCs with 1,2-benzenedithiol, was used to quantify the uptake of ITCs in blood humans [85]. After eating a total of 350 g of cooked watercress About one-third of phenethyl ITC (PEITC) was emitted as PEITC-NAC compared with uncooked watercress. These indicate results that bioavailability of PEITC is markedly compromised by cooking. That the cooked watercress is completely devoid of myrosinase action for hydrolysis of glucosinolates to ITCs and yet ITC metabolites were still set up in urine, likely to that microflora be concerned in converting gluconasturtiin to PEITC after in intestinal after ingesting cooked watercress (Figure 4). The mercapturic acid pathway was stated by Meskin *et al.* [86].

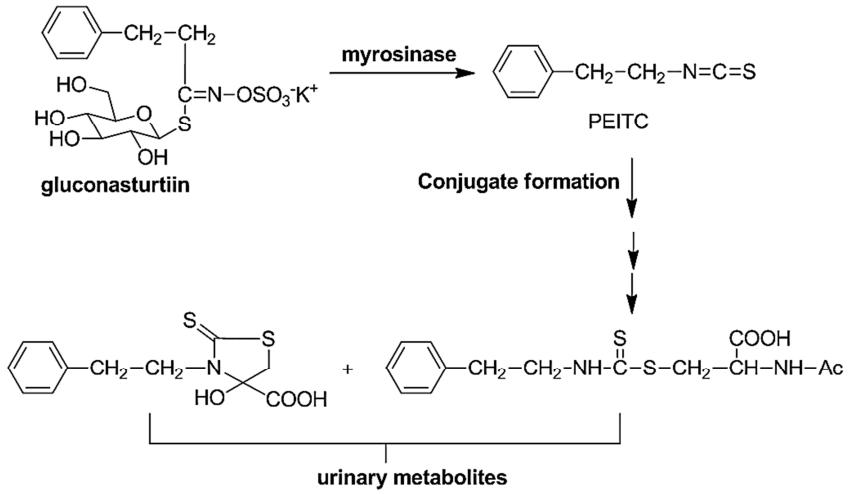

Figure 4. *Metabolism of gluconasturtiin and PEITC in rodents and humans. Hydrolysis of gluconasturtiin by myrosinase to PEITC shadowed by GSH conjugation and enzymatic degradation via the mercapturic acid pathway.*

Benzoquinones, phenolic acids, phenyl acetic acids, and phenyl propene's to coumarins and isocoumarins, naphthoquinones, and anthraquinones to higher forms are Polyphenols establish another family of plant compounds that variety from simple phenols, such as flavonoids and lignin's, and to highly polymerized compounds, such as bioflavonoids, proanthocyanidins, or condensed tannins with molecular weights greater than 30,000 Dalton [87].

Because tannins bind to the protein and decrease digestibility, have polyphenols been considered anti-nutrients [85]. Though, the phenolic have substantiated to be very good antioxidants, rummaging free radicals and delivering metal chelating activities [88].

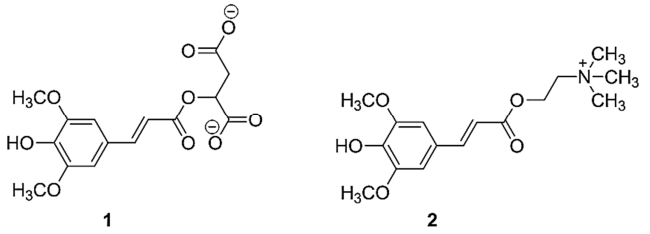

Figure 5. *Structures of sinapoyl malate (1), and sinapoyl choline (2).*

Also, cinnamic esters are discovered as sugar esters, or as esters of a multiplicity of other organic acids. For example, sinapoyl esters symbolize a class of UV-absorbing complexes in the family of the *Brassicaceae*. Examples consist of sinapoyl malate (1) present in leaves and sinapoyl choline (2) (Figure 5) present in roots [89]. *Eruca sativa* is a member of the *Brassicaceae*, which is considered to be an important chemo-preventive plant family.

Total polyphenols results exposed an increase in experimental parameters when associated with the control sample, where they amplified by varying degrees, as they were as follows *Fusarium oxysporum* (T1); *Macrophomina phaseolina* (T2); *Sclerotinia sclerotiorum* (T3); *Trichoderma longibrachiatum* (T4); *Alternaria alternate* (T5); *Penicillium digitatum* (T6), and Cu SO_4 (T7), 0.67, 22.00, 80.0, 54.13, 122.68, and 64.80%, respectively (Table 3). Although total flavonoids generally took the same trend in the increase in experimental treatments for control treatment, the highest coefficients gave the highest percentage increase in total polyphenols was lower in flavonoids compared to control, +23.48% and -78.69% in T3 and T5, respectively. In addition, total flavonoids of T1, T2, T4, T6, and T7 were higher by about 99.83, 92.11, 221.14, 280.70, and 224.49%, respectively, compared with those in the control treatment (Table 3).

Table 3. *Total polyphenols and total flavonoids content of rocket meal seed (RMS) in different treatments.*

Sample (Treatment)	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days)	83.33	5.96
T1: Control + *Fusarium oxysporum*	83.89	11.91
T2: Control + *Macrophomina phaseolina*	101.67	11.45
T3: Control + *Sclerotinia sclerotiorum*	150.00	7.36
T4: Control + *Trichoderma longibrachiatum*	128.44	19.14
T5: Control + *Alternaria alternata*	185.56	1.27
T6: Control + *Penicillium digitatum*	148.89	22.69
T7: $CuSO_4$	137.33	19.34

The most general vegetables expended all over the creation are *Brassicaceae* plants and to be a moral source of bioactive phytochemicals. *Brassica* species and varieties are increasingly flattering a examine perfect in plant science, as a sign of the consequence of their major and secondary metabolites. Plant interaction with environmental stress factors counting animals and beetles herbivore, pathogens, metal ions, bright, is known to lead to the encouragement of various defense apparatuses resulting in a qualitative and/or measureable change in plant metabolite production. Pre-harvest and/or post-harvest situations affect this since plants produce gesticulating molecules that cause a direct or indirect activation of metabolic pathways. That ultimately affects the production of phytochemicals, such as carbohydrates (sucrose and glucose), amino acids, phenolics (phenylpropanoids and flavonoids), and glucosinolates. These phytochemicals have diverse suggestions due to their antimicrobial, antioxidant and anti-carcinogenic properties, but on the other hand, these compounds or their breakdown commodities can act as anti-nutritional factors in the diet. In this report a wide range of the stress-induced metabolic responses in the *Brassica* plants commonly used for human consumption [90]. Table 4 shows the reduction power of rocket seed meal treatments. The results of four treatments T1, T3, T5, and T7 were lower than the control treatment while three other treatments T2, T4, and T6 were greater than the control treatment. Top transactions are T2 (25.98%), T4 (104.85%), and T6 (63.54%) compared with those in the control treatment. The lowest transactions are T1 (46.36%), T3 (44.07%), T5 (7.90%), and T7 (41.71%) compared with those in the control treatment.

Table 4. *Reducing power of rocket seed meal treatments.*

Sample (treatment)	Optical density at 700 nm
Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days)	1.443
T1: Control + *Fusarium oxysporum*	0.774
T2: Control + *Macrophomina phaseolina*	1.818
T3: Control + *Sclerotinia sclerotiorum*	0.807
T4: Control + *Trichoderma longibrachiatum*	2.956
T5: Control + *Alternaria alternata*	1.329
T6: Control + *Penicillium digitatum*	2.360
T7: $CuSO_4$	0.841

The antioxidant motion of compounds was overturned: quercetin > cyanidin > catechin In the aqueous-based ABTS+ method [91]. Buchwaldt *et al*. [92] inaugurate that on Czapek-Dox medium, sinigrin (allyl-GLs) does not stimulation growth degrees of *Alternaria brassicae*, but at mounting singrin concentrations, colonies become murkier on potato dextrose agar (PDA), colonies are dark at all singrin concentrations. Table 5 shows the antioxidant capacity of treatments determined by (ABTS+) action radical. Three were higher value coefficients, while the other four were lower than in the control transaction. Top transactions are T2, T4, and T6 by about 25.98, 104.85, and 63.54%, respectively, While the lowest transactions are T1, T3, T5, and T7 by about 46.36, 44.07, 7.90, and 41.71%, respectively, compared with those in the control treatment (Table 5).

Table 5. *Antioxidant capacity of treatments determined by ($ABTS^{+}$) action radical.*

Sample (Treatment)	% Inhibition
Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days)	28.86
T1: Control + *Fusarium oxysporum*	15.48
T2: Control + *Macrophomina phaseolina*	36.36
T3: Control + *Sclerotinia sclerotiorum*	16.14
T4: Control + *Trichoderma longibrachiatum*	59.12
T5: Control + *Alternaria alternata*	26.58
T6: Control + *Penicillium digitatum*	47.2
T7: $CuSO_4$	16.82
+Ve Control (Ascorbic acid)	91.41
-Ve Control	0

Recently, twilight primrose meal and phenolic components of borage were cognizant by Wettasinghe and Shahidi [93, 94]. Evening primrose controlled gallic acid, (+) catechin and (-) epicatechin, and a high-molecular-weight polyphenol known also nothin B, while Borage meal confined rosmarinic acid, syringic acid, and sinapic acid [95]. The nothin B compound has also been isolated from leaves and twigs of evening primrose as well as other plants [96]. This complex was an effective antitumor genic and anti-carcinogenic agent [97]; it's impending health-promoting activity. The sensitive oxygen species - and 2,2-diphenyl-1-picrylhydrazylradical (DPPH) - evening primrose extracts have been documented and scavenging activity of borage [98]. Dietary in rats evening primrose was also found to have a hypocholesterolemic effect [99]. Table 6 shows the antioxidant commotion using (DPPH) radical. Five coefficients were higher, while the other two were lower in comparison to control. Top transactions are T1, T2, T3, T4, and T6 by about 9.27, 42.78, 13.57, 342.72, and 9.93, respectively, While the lowest transactions are T5, and T7 by about 2.90 and 22.84, respectively, compared with those in the control treatment (Table 6).

Table 6. *Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical of Antioxidant activity.*

Sample (Treatment)	% Inhibition
Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days)	1.650
T1: Control + *Fusarium oxysporum*	1.803
T2: Control + *Macrophomina phaseolina*	2.356
T3: Control + *Sclerotinia sclerotiorum*	1.874
T4: Control + *Trichoderma longibrachiatum*	7.305
T5: Control + *Alternaria alternata*	1.602
T6: Control + *Penicillium digitatum*	1.814
T7: $CuSO_4$	1.273

Phenolic during their autoxidation in a process that is dependent on divalent metal ions by Hydrogen peroxide (H_2O_2), these activities may be due to the concomitant construction of comparable to the convinced pro-oxidant effect of vitamin C, that polyphenols are redox chemicals [100, 101]. Also, Hydrogen peroxide is pondered as a Reactive Oxygen Species (ROS) because of its reactivity [102]. Table 7 shows results the hydrogen peroxide scavenging activity of samples rocket seed meal laboratories. In the same direction as the results of the previous chemical measurements in the study, three coefficients were higher than the control transaction value, while the other four experimental treatments were less than the control transaction value. And in terms of 25.98, 104.85, 63.54% and on the order of transactions T2, T4, and T6 were higher compared with those in the control treatment, while the lowest transactions are T1, T3, T5, and T7 by about 46.36, 44.07, 7.90, and 41.71%, respectively, compared with those in the control treatment (Table 7).

Table 7. *Hydrogen peroxide (H_2O_2) scavenging activity of samples E. sativa meal laboratories.*

Sample (Treatment)	H_2O_2 scavenging activity%
Control (1 meal: 3 water ratio, at Twenty-five degrees Celsius under aerobic condition, ten days)	14.43
T1: Control + *Fusarium oxysporum*	7.74
T2: Control + *Macrophomina phaseolina*	18.18
T3: Control + *Sclerotinia sclerotiorum*	8.07
T4: Control + *Trichoderma longibrachiatum*	29.56
T5: Control + *Alternaria alternata*	13.29
T6: Control + *Penicillium digitatum*	23.6
T7: $CuSO_4$	8.41
+Ve Control (Ascorbic acid)	45.70
-Ve Control	0

Reactive oxygen species (ROS) have living organisms industrialized various ways to deal with. One mechanism is enzymatic inactivation. catalyzes the contention of superoxide into oxygen (O_2) via oxidation and (H_2O_2) via reduction in the enzyme superoxide dismutase, (E.C.1.15.1.1) "This is the recommended number of the enzyme". is indifferent through the action of E.C. 1. 11.1.6 (the enzyme catalase), and E.C.1.11.1.9 (glutathione peroxidase) the H_2O_2, which is reactive itself. Catalase catalyzes the conversion of H_2O_2 to water and oxygen, whereas glutathione peroxidase catalyzes the formation of oxidized glutathione (G-S-S-G) from reduced glutathione (G-SH), at the expense of H_2O_2 [102].

4. Conclusions

This paper mainly studied on the physiological response of fungi to ITCs using the solid state fermentation and copper sulfate solution methods for glucosinolates detoxification. The topic of the paper is meaningful and the results of the study have some reference values for the related fields. The influence of Gls metabolites current in animal yield on human health also needs to be investigated. From the present revision it appears that approving a suitable Gls detoxification technology, *E. sativa* meal can commendably use in animal feed preparations. The results of this study showed that there are three biological factors which are T2 (*Macrophomina phaseolina*), T4 (*Trichoderma longibrachiatum*), and T6 (*Penicillium digitatum*) achieved the highest ratios that exceed the control treatment (Non-biologically and chemically treated), in the measurements of total polyphenols and total flavonoids content, Reducing power, ($ABTS^{+}$) action radical, (DPPH) radical, and Hydrogen peroxide (H_2O_2) scavenging activity of rocket meal seed (RMS) in different treatments. Glucosinolates is analyzed by hydrolysis of the enzymatic enzyme myrosinase system, which is produced by many fungal cells and the production of many chemical compounds with varying effects between the beneficial and harmful. There is a positive role for these toxic products from GLS hydrolysis, especially in the defense of plants that contain coating against fungal pathogens. Biocidal action against a wide variety of organisms for example insects, plants, fungi and bacteria of GLS hydrolysis products, So recommend more studies that classify these products, isolate each product individually, and study its biological effects, individually and collectively. Genetic scientists have been able to identify the genes in the secretion of hydrolysis enzymes with various fungi to remove and break down toxins, as well as genes controlling the use or non-utilization of by-products of plant and fungal metabolism. Further studies are recommended in the field of genetic engineering to produce vehicles beneficial to humans and animals Directly, the plants and their offspring are transformed from food and may be harmful or toxic, to food and medicine for treatment and before it for prevention requires further attention.

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