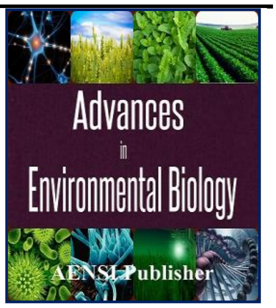
Advances in Environmental Biology, 9(23) October 2015, Pages: 361-367

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/



Auxin-like and Gibberellic acid- like activity of Pleurotus sajor-caju (Fr.) Singer and Volvariella volvacea Fr. on Tomato (Lycopersicon esculentum Mill.) Seedlings

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ARTICLE INFO

Article history:
Received 28 September 2015
Accepted 30 October 2015
Available online 24 November 2015

Keywords:
auxin; phytohormones; germination; gibberellic acid; indole acetic acid; root initiation; stem elongation

ABSTRACT

In the Philippines, *Pleurotus sajor-caju* (Fr.) Singer and *Volvariella volvacea* Fr. are among the most commonly utilized and cultivated mushrooms. They can also be found growing in various agricultural wastes. Various researches has already been conducted on the medicinal, nutraceutical as well as neutraceutical properties of mushroom but little has been reported on their ability to produce phytohormones which stimulates the growth of various plants. This study aimed to evaluate the potential of *P. sajor caju* and *V. volvacea* hot water extracts in producing auxin-like activity by stimulating root initiation in tomato seedlings. Meanwhile, their gibberellic acid- like activity was evaluated through seed germination and stem elongation. Hot water extracts was prepared by mixing 100 g of powdered fruiting bodies in 500 mL distilled water and for the positive control, 10^{-2} M stock solution of Indole Acetic Acid and Gibberellic Acid was prepared. Application of *P. sajor-caju* and *V. volvacea* hot water extracts on tomato seeds resulted to 35.7% and 17.9% increased in seed germination. For stem elongation, extracts of *P. sajor-caju* resulted to 79% increased in the length of stem and *V. volvacea* with 59.8%. In addition, potential auxin-like activity was indicated in the study wherein no significant difference was obtained in *V. volvacea* and indole acetic acid -treated seedlings. The mean number of roots initiated by seedlings treated with *V. volvacea* and *P. sajor caju* was also comparable to commercial IAA with 35.2, 31.5 and 37.1 respectively. Based on the results of the study, it can be concluded that both *P. sajor-caju* and *V. volvariella* hot water extracts exhibits auxin like- activity and gibberillic acid activity in tomato seedlings. Thus, the study revealed the potential of *P. sajor-caju* and *V. volvacea* as sources of phytohormones.

To Cite This Article: Mary Jhane G. Valentino and Cecilia T. Galvez., Auxin-like and Gibberellic acid- like activity of Pleurotus sajor-caju (Fr.) Singer and Volvariella volvacea Fr. on Tomato (Lycopersicon esculentum Mill.) Seedlings. ***Adv. Environ. Biol., 9(23),*** *361-367, 2015*

INTRODUCTION

Mushrooms are fleshy spore bearing saprophytic macrofungi growing on various agricultural wastes, damp rotten log of wood trunk of trees, decaying organic matter and in damp soil rich in organic substances [1]. Mushrooms exist in various varieties and about 2000 species of mushrooms exist in nature.In recent years, many scientists are interested in their complex and varying morphology, chemical composition, and their biological activity [2, 3]. They contain high amount of dietary fibers, protein, vitamin B groups (thiamin, riboflavin, folic acid and niacin), vitamin D, beta glucan and other important minerals. In fact, several researches revealed the potentials of mushrooms which can be attributed to various enzymes they produce. These include their antitumor activities, antimicrobial activities, immunomodulating potential, hypocholesteroiaemic activity, antioxidant properties [4, 5, 6, 7, 8]. Moreover, mycoremediation activity as well efficiency in recycling has already been proven through mushroom biotechnology [9].

The *Pleurotus* genus of the class Basidiomycetes belongs to a group known as “white rot fungi” or oyster mushroom since they produce a white mycelium and degrade lignin and cellulose. *Pleurotus* ranked second among the cultivated mushrooms in the world [10, 11, 12]. In addition, they are also known for different cellulolytic, amylolytic enzymes and protease activity [13, 14, 15]. Polysaccharides synthesized by *Pleurotus*, including the β-glucans, are considered primarily responsible for their therapeutic properties [16]. On the other

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hand, *Volvariella volvacea* is known as the oil palm or paddy straw mushroom belonging to Pluteaceae family and is the third most important cultivated mushroom in tropical, subtropical regions and temperate regions of both the eastern and the western hemispheres [17, 18]. Furthermore, Ramkumar [19] and Kalava and Menon [20] reported that *V. volvacea* contain catalase, superoxide dismutase, glutathione peroxidase, peroxidase, glutathione-S-transferase and glutathione reductase and they can also accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Moreover, studies have been conducted on the isolation of phytohormones in edible mushrooms.

Phytohormones are substances produced by plants which play vital roles in assimilating many aspects of plant growth and development. They can promote, inhibit or modify physiological and morphological processes when applied at different concentrations [21]. They are classified as auxins, gibberellins, cytokinins, ethylene, brassinosteroids, strigolactones, jasmonic acid, salicylic acid and polyamines [22].

Auxin plays important roles during the entire life span of a plant. This small organic acid influences cell division, cell elongation and cell differentiation, and has great impact on the final shape and function of cells and tissues in all higher plants [23]. In addition, auxin was initially identified as the bioactive substance that induces roots in plant tissue culture [24]. Meanwhile, Gibberellic acid is a tetracyclic di-terpenoid compound, responsible for stimulating plant growth and development. It is responsible in seed germination, meristem to shoot growth, juvenile to adult leaf stage, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors [25].

Hence this study was conducted to assess the auxin-like and gibberellic-activity of the selected mushroom which would further lead to the utilization and isolation of its bioactive components of mushrooms as sources of expensive phytohormones necessary for the micropropagation of other important crops.

MATERIALS AND METHODS

The gibberellic acid and auxin-like activity of *P. sajor-caju* and *V. volvacea* was evaluated through their effect on stem elongation, seed germination and root initiation in tomato plants. For the positive control, 10^{-2} M stock solution of Indole Acetic Acid and Gibberellic Acid was prepared.

Preparation of hot-water extracts:

One hundred grams of dried and powdered fruiting bodies of *P. sajor caju* and *V. volvacea* were used for the hot water extract preparation. One hundred grams of powdered fruiting bodies were mixed with 500 mL of distilled water in an Erlenmeyer flask. The mixture was placed in water bath at 80–90 $^{\circ}$C for 2 hours. Then, the mixture was filtered with Whatman filter paper No.1 for the extract solution. The filtrate was poured in a sterile amber bottle and was placed in the refrigerator until needed.

Preparation of soil medium:

Garden soil, organic fertilizer and rice hull ash were properly mixed in 1:1:1 ratio and was placed in planting bags.

Bioassay for Gibberellic acid activity:

Seed germination:

Viability test was carried out prior to the test by soaking tomato seeds in distilled water for two hours. Floating seeds were discarded and only the viable seeds were used

For seed germination test, viable seeds were subjected to different treatments. Tomato seeds were placed in Petri plates lined with sterile tissue paper. Then each plate was flooded individually with 5 ml of different treatments. The number of seeds germinated was counted after 3 days of incubation at 28 $^{\circ}$C.

Stem elongation:

Two week old tomato seedlings were soaked into each treatment for 30 minutes and then they were planted in the soil medium. Each seedling was sprayed with different treatments on the stem apex of the tomato seedlings with an interval of 48 hrs. The initial and the final length of the stem of each tomato seedlings were measured in cm after 25 days of cultivation.

Bioassay for auxin like activity:

Root initiation:

Auxin exerts control over root formation. For this test, each seedling was cut near the base of the stem and was soaked in each treatment for 30 minutes. After which, the seedlings were planted in seedling bags with soil medium. Each seedling was treated with each treatment daily (7am and 5pm). The number of roots initiated was counted after 25 days of cultivation.

Statistical analysis:

Data were analyzed using ANOVA at 0.05 level of significance and Comparison among means using the Least Significant Difference.

RESULTS AND DISCUSSION

Gibberellic acid like activity:

Gibberellic acid was first discovered in 1954 from an ascomycetes *Gibberella fujikuroi* that was later identified as *Fusarium heterosporium* [26]. It functions in stimulating stem elongation of dwarf plants, seed germination, breaking of seed dormancy, flowering, fruiting, leaf growth, and cell division and growth [27, 28].

Table 1 presents the gibberellic acid activity of *P. sajor caju* and *V. volvacea*. Results for seed germination showed that seeds treated with commercial gibberellic acid had the highest mean number of germinated seeds of 4.2 followed by *P. sajor caju*-treated seeds of 3.8 and *V. Volvacea* - treated seeds of 3.3. An increase of 50% in seed germination was observed when treated with commercial gibberellic acid ,while seeds treated with *P. sajor caju* and *V. volvacea* extracts had 35.7% and 17.9% increase in the number of seeds germinated, respectively. Also, statistical analysis revealed significant differences among the treatment means. This suggests the potential of *P. sajor caju* and *V. volvacea* in stimulating seed germination in tomato.

Results of the study coincide with the findings of several studies [29, 30, 31, 32, 33, 34, 35, 36, 37] illustrating the functions of gibberellic acid in seed germination. This hormone stimulates the seed germination by increasing the growth potential of embryo and inducing hydrolytic enzymes. Wherein, during seed germination embryo produces gibberellic acid which influences aleurone cells to produce hydrolytic enzymes such as α- and β-amylase that hydrolyze starch to glucose. It also affects the proteins that produce mRNA and thereby increases DNA replication and induces analysis of endospermic materials in the seed.

Stimulation of seed germination by *P. sajor caju* and *V. volvacea* can be due to the presence of polyphenols in mushrooms. Phenolic compounds in mushrooms was accumulated as end products of shikimate and acetate pathways [38]. Evidente *et al*. [39] revealed that polyphenols can stimulate *Orobanche* and *Phelipanche* species seed germination. *P*- benzoic acid, *p*-phenyl aetic acid, *o*-coumaric acid, ferulic acid and chrysin were among the most common polyphenols found edible mushroom extracts [40].

Table 1: Gibberellic Acid like Activity of *P. sajor-caju* and *V. volvacea* on tomato (Seed germination).

TREATMENTS	Seed Germination Mean number of seeds germinated	Percentage increase in mean number of seeds germinated (%
Distilled water(- control)	2.8d	
Gibberellic acid(+control)	4.2a	50.0
P. sajor caju	3.8b	35.7
V. volvacea	3.3c	17.9

* Treatment means with the same letter are not significantly different at 0.05 level of significance

As presented in table 2, evaluation on the stem elongation resulted to an evident increase in the height of the tomato seedlings. Commercial gibberellic acid-treated seedlings had the highest acceleration with 44.9 cm which is 100% increase in stem elongation as compared to untreated seedlings. Meanwhile, *P. sajor-caju* – treated seedlings and *V. volvacea* – treated seedlings with 40.1 cm and 35.8 cm respectively, leading to about 79% and 59.8% increase in the height of the seedlings. Statistical analysis indicated that *P. sajor- caju* is comparable to commercial gibberellic acid while *V. volvacea* is also comparable to *P. sajor caju*. These provide evidence that the mushroom extracts greatly affects the growth of the tomato seedlings in terms of stem elongation.

Table 2: Gibberellic Acid like Activity of *P. sajor-caju* and *V. volvacea* on tomato (Stem elongation).

TREATMENTS	Stem elongation Mean increase in length of stem (cm)	Percentage increase in stem elongation (%)
Distilled water(- control)	22.4d	
Gibberellic acid(+control)	44.9a	100
P. sajor caju	40.1ab	79.0
V. volvacea	35.8c	59.8

* Treatment means with the same letter are not significantly different at 0.05 level of significance

Results of the study can be attributed to the functions of gibberellic acid in cell division and cell elongation which can promote elongation of the internodes. In addition, it can also influence the increase of cell membrane permeability which facilitates the utilization and absorption of nutrients and transport assimilates [41, 42, 43].

This also conform with the reports of Sasaki *et al*. [44] and Gemeci *et al*. [45] wherein the application of gibberellic acid on tomato plants showed significant increase in stem length, seed germination fruit size and indicated that 10 ppm gibberellic acid- treated tomato plants showed a 17% increase in stem length, it is also effective in increasing fruit size and that single applications of very small amount of gibberellic acid to seeds or seedling roots changed the growth rates of leaves and trusses.

Gibberellic acid is a terpenoid produced in different parts of the plant from the root tips to the developing grains but the shoots and the young leaves at the tip of the branches are considered the activation sites. Its highest production is at the time of cell division and its transmission is done within organs [21, 22]. Thus, the significant increase in seed germination and stem elongation of the tomato seeds and seedlings treated with mushroom extracts can be attributed to the presence of terpenoids in the *P. sajor caju* and *V. Volvacea*. Accordingly, studies revealed that biologically active compounds present in extracts of *P. sajor-caju* are mainly low molecular weight such as lectins, triterpenoids and fatty acids [46]. In addition, Nieto and Chegwin [47] had isolated 10 triterpenic compounds from *P. sajor caju*. While three new triterpenoids, sublateriols A-C (4-6), were isolated from *Naematoloma sublateritium* [39]. Finally, it was reported that terpenoids exhibits a wide range of biological activities such as antibiotics, anti-inflammatory, anti-HIV and anti-tumor effects, hypotensive agents, sweeteners, insecticides, anti-feedants, phytotoxic agents, perfumery intermediates and plant growth hormones [48,49] .

Auxin like activity:

Auxin is the first plant hormone to be discovered which is primarily involved in cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins. Auxin is synthesized in meristematic regions and other actively growing organs such as coleoptiles, apices, root tips, germinating seeds, and the apical buds of growing stems [50, 51, 52]. As revealed by Ahmad [53], microorganisms produce auxin by utilizing the substrate of plant exudates containing L-tryptophan. In addition, they could promote root growth either directly by stimulating plant cell elongation or cell division or indirectly by its influence on the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [54]. Also, IAA regulates many plant developmental processes including embryogenesis, root and stem elongation, phyllotaxy, apical dominance, photo- and gravitropism, and lateral root initiation [55].

Results of the present study showed observable increase in the number of roots initiated. As shown in Table 3, tomato seedlings treated with commercial IAA had the highest mean number of roots of 37.1 (36.4%) followed by *V. volvacea* - treated seedlings and *P. sajor caju*–treated seedlings, with 35.2 (29.4%) and 31.6 (16.2%) respectively. In addition, statistical analysis revealed significant differences between the untreated seedlings and the seedlings treated with mushroom extracts (*P. sajor caju* and *V. volvacea*). Moreover, seedlings treated with commercial gibberellic acid and *V. volvacea* are comparable to one another. Results depict the auxin like activity of mushroom extracts in terms of root initiation.

Application of exogenous auxins increases concentration of natural auxins in cuttings. Several studies revealed that mushroom species have the ability to produce IAA [56]. The auxin formation in a submerged culture of the xylotrophic basidiomycete *Lentinus edodes* was studied and biologically active substances of an indole nature are identified which stimulates the mycelia growth and induction of tryptophan-independent paths of auxin biosynthesis [57].

Also, Bose *et al*. [58] revealed the ability of three white rot fungi (*Trametes versicolor*, *Pleurotus ostreatus*, and *Phanerochaete chryosporium*) to produce Indole Acetic Acid (IAA) when incubated with tryptophan. Also, several studies [59, 60, 61, 62] reported exogenous IAA production by *Phanerochaete chrysosporium* strain, *Funalia trogii* and *Lentinus sajor-caju*, respectively. While, Unyayar *et al*. [63] evaluated the abscisic acid production by white rot fungi. In addition, Crocoll *et al*. [64] demonstrated *Funalia trogii* and *Trametes versicolor* to produce gibberellic acid, abscisic acid, indole acetic acid, and cytokinin employing olive oil mill and alcohol factory wastewaters as raw materials for fermentation.

Table 3: Auxin like Activity of *P. sajor-caju* and *V. volvacea* on tomato.

TREATMENTS	Root initiation Mean number of roots initiated	Percentage increase in number of roots inititiated (%)
Distilled water(- control)	27.2d	
Indole acetic acid (+control)	37.1a	36.4
P. sajor caju	31.6c	16.2
V. volvacea	35.2ab	29.4

* Treatment means with the same letter are not significantly different at 0.05 level of significance

The study revealed the potentiality of *P. sajor-caju* and *V. volvacea* in exhibiting auxin and gibberellic acid like functions in seed germination, stems elongation and root intitiation of tomato. Thus, this could lead further to the isolation and utilization of its bioactive compounds in search for alternatives for expensive phytohormones that are primarily used in micropropagation of crops.

Conclusion:

Based on the results of the study, *P. sajor caju* and *V. volcecea* hot water extracts exhibited a potential auxin-like and gibberellic acid like activity on tomato seedlings. This was exhibited by stimulating stem elongation, seed germination and root initiation of the tomato plants. Thus, the study indicates the potentiality of the two selected mushroom as sources of auxin and gibberellic acid which is vital in the micropropagation of important crops.

ACKNOWLEDGMENT

To God be the Glory. Thy will be done!

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