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# EFFECT OF IRRADIATION AND STORAGE ON THE CYTOKININS OF EDIBLE MUSHROOM'S STIPE

M. Wahid

*Nuclear Institute for Food and Agriculture Tarnab, Peshawar*

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Freshly harvested mushrooms were irradiated (0 and 2.5 kGy) and stored for 6 days at 14 ± 2°. The samples were studied for the growth and cytokinin activity in the upper and lower portions of mushroom's stipe separately. Upper portion of the stipe elongated more than the lower portion during storage. Cytokinin activity was higher on 1 day than 0 day and then decreased during storage. Upper portion of stipe contained higher cytokinin contents than the base. Irradiation strongly inhibited the growth and cytokinin activity in both the investigated portions of mushrooms's stipe.

## INTRODUCTION

The fruit body of *Agaricus bisporus* is composed of three main regions, the cap, gills and stipe. The cap protects the gills surface which ultimately produce the spores, and stipe serves to raise these regions into a position suitable for the disposal of basidospores.

There is a general statement that in various basidopores the upper portion of the stipe expands to a much greater extent than the base [1]. The biochemical basis of stipe elongation is very poorly understood and most studies have been concentrated on aspects of wall synthesis. Gooday [2] found that the elongation of stipe was accompanied by a considerable increase in chitin contents. There was a low content of chitin synthase activity in the stipe at the end of elongation [3]. Cytokinins are known to be involved in all phases of plant growth and development [4]. The present studies were undertaken to investigate the expansion of stipe on the basis of cytokinin activity in mushrooms. The effects of gamma irradiation on the growth rate and cytokinin activity also were considered.

## MATERIALS AND METHODS

*Agaricus bisporus* (L.) variety of mushroom, used in the present investigation, was obtained from the mushroom production unit of DUNA Agricultural Cooperatives, Budapest, Hungary. The fresh mushrooms were harvested at the stretched veil stage, having a dia of 40-45 mm. Radiation treatment (2.5 kGy dose) was carried out in the $^{60}Co$ gamma irradiation source of 45 kCi activity at Central Food Research Institute, Budapest. Irradiated and unirradiated samples were stored at 14 ± 2° with 80–90% relative humidity. The stored samples were examined for the growth rate of stipe, and changes in cytokinin activity for 0, 1, 2, 4 and 6 days.

*Elongation of Stipe.* The samples were sorted for their uniform shape and size and divided into two lots of 10 mushrooms each for 0 and 2.5 kGy treatments. A portion of the cap of fruit body was removed (V shaped) to expose the top of the stipe. The length of the stipe varied from 40–42 mm and the measurement was made using varnier caliper. Whole length of the stipe was equally divided into two portions and marked with Indian ink. Stipe length (in mm) of the marked fresh portions and changes in their lengths were carefully observed till 6th day of storage. The increase in relative growth was calculated from the length of the stipe before and after expansion and expressed in percent.

*Extraction and Determination of Cytokinin.* Upper and lower portions of mushroom's stipe were cut with stainless knife. Each portion of the stipe (100 g) was blended separately for 5 min with 80% ethanol three-times the quantities of the sample [5]. The homogenate was kept in refrigerator overnight and then filtered. The filterate was evaporated under vacuum at 40° and set at pH 3.0. The concentrate was shaken with 4 volumes of ether. The ether phase, being cytokinin inactive, was not used. The aqueous phase was set at pH 6.5, and then shaken with 4 volumes of n-butanol saturated with water. The butanol phase was eva-

porated under vacuum at 40°. The extract was purified on an insoluble polyvinylpyrrolidone (PVP) column according to the method of Biddington and Thomas [6]. The column flow rate was 36 ml/hr. The cytokinin elution profile was obtained by monitoring the transmittance of UV light at 254 nm through the elute using a LKB Uvicord II absorbance meter.

*Amaranthus betacyanin* bioassay was adopted for the rapid determination of cytokinins. This method is based on the cytokinin induced formation of the betacyanin in the cotyledons and hypocotyl of *Amaranthus* seedlings, incubated in darkness, in the presence of tyrosine. Ten seedlings, without seed coat, of *Amaranthus paniculatus* (pigmitorch vetomag Co. Budapest) were used for each determination. Betacyanin was extracted with two or three cycles of thawing and freezing. The quantities were determined by calculating the difference between optical densities at 542 and 620 nm [7] and comparing the values with standard graph.

## RESULTS AND DISCUSSION

The increase in relative growth in the upper and lower portions of mushroom's stipe is illustrated in Fig. 1. It was observed that there was a considerable increase in the length of the unirradiated stored mushroom's stipe. The growth rate was linear for the first two days and then attained a maximum limit on the 4th day of storage. Upper portion of stipe had significantly ($P < 0.01$) higher growth rate as compared to the lower portion. The growth pattern was similar in both the cases. Visual observations of the individual samples revealed that both the parts of stipe ceased growing almost at the same time. Radiation dose of 2.5 kGy completely inhibited the growth in both the investigated portions of the stipe.

Eilers [8] has shown that the upper two-third of *C. radiatus* was responsible for 80–90% of the total stipe expansion. Similar results have been obtained by Cox and Niederpruem [9] in a study of stipe elongation in *C. cinercus*. They pointed out, however, that maximum expansion was localized in the upper mid region than the extreme top of the stipe. Craig and Gull [1] also reported that the expansion of the stipe occurred mainly in its upper portion. The results of the present study corroborate the earlier findings.

Cytokinin activity of both upper and lower portions of stipe was determined by the biotest of *Amaranthus betacyanin*. About 25 fractions obtained by LKB apparatus was tested in each case. The results are expressed in zeatin

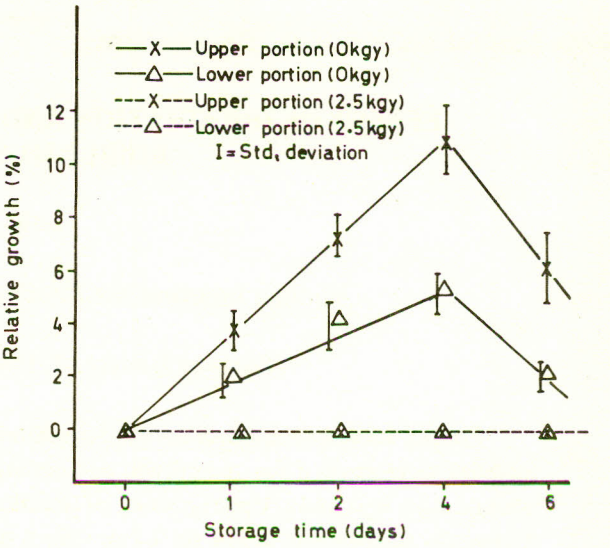


Fig. 1. Relative growth of upper and lower portions of mushroom's stipe in function of irradiations and storage (Temp. 14±2°C, R.H. 80–90%).

equivalents and presented in Table 1. Betacyanin production in the upper and lower portions, in the presence of cytokinin extracts, of fresh (0 day) mushroom's stipe is illustrated in Fig. 2. The results of investigation revealed that no activity of cytokinin was observed in the first five fractions which were collected from the upper and lower portions of mushroom's stipe. Less activity of cytokinin ($P < 0.01$) was exhibited by the fresh (0 day) upper and

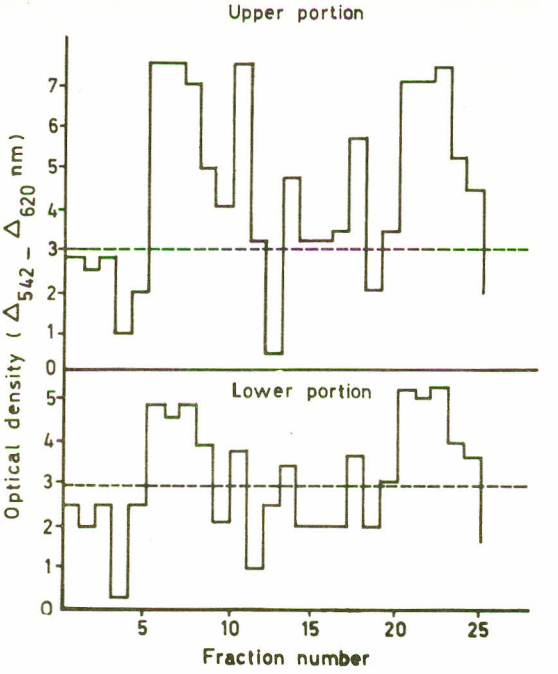


Fig. 2. Betacyanin production in *Amaranthus paniculatus* in the presence of cytokinin extracts of fresh mushroom's stipe.

Table 1. Changes in cytokinin activity of mushrooms' stipe (Expressed as zeatin ng $kg^{-1}$ sample)

| Dose (kGy) | Stipe's portion | | Storage time (days) 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| 0 | Upper | $\bar{x}$ | 885 | 1953 | 781 | 210 | 0 |
| | | s | ± 50 | ± 70 | ± 48 | ± 20 | 0 |
| | Lower | $\bar{x}$ | 465 | 623 | 373 | 90 | 0 |
| | | s | ± 70 | ± 35 | ± 60 | ± 15 | 0 |
| 2.5 | Upper | $\bar{x}$ | 876 | 0 | 0 | 0 | 0 |
| | | s | ± 62 | 0 | 0 | 0 | 0 |
| | Lower | $\bar{x}$ | 456 | 0 | 0 | 0 | 0 |
| | | s | ± 62 | 0 | 0 | 0 | 0 |

$\bar{x}$ Sum of 1-25 cytokinin fractions (Average of 3 replications; s = Standard deviation

lower portion of the stipe than those of 1 day stored samples. A decreasing trend in activity was observed, thereafter, upto 6 days of storage. It was also noted that the upper portion of the fresh (0 day) stipe contained significantly higher quantities of cytokinin ($P < 0.01$) as compared to the lower portion (Fig. 2) and this difference remained the same throughout the storage period. No immediate effect of irradiation was observed in the upper and lower portions of the stipe at 0 and 2.5 kGy doses. Many of the cytokinin fractions obtained from fresh 2.5 kGy treated samples showed activities which nearly equalled that of unirradiated (0 kGy) samples. Radiation dose of 2.5 kGy, however, completely inhibited the cytokinin activity during storage as shown in Table 1.

The data obtained in the present investigation indicated that fresh stipe portions contained higher cytokinin contents than the stored samples. Similar results of cytokinins have been reported for corn and tomatoes during storage [10,11]. Upper portion of mushroom's stipe elongated to a considerable extent and contained higher quantities of extracted cytokinins than the base portion. Since the high cytokinin activity is usually associated with cell division [4,12], therefore, higher amounts ($P < 0.01$) of cytokinins probably resulted in conditions of active cell division and consequent elongation of the upper portion more ($P < 0.01$) than the base of the stipe. The ionizing radiation induced changes on the growth have been ascribed to changes mainly in auxin activity in plant tissues [13,14,16]. Redel *et al.* [15] reported that radiation effects could also be related to the cytokinin mediated growth inhibition. It was observed during these investigations that the irradiation treatment (2.5 kGy) suppressed the cytokinin activity which resulted in the inhibition of growth in mushroom's stipe. Kovacs and Vorös [11] observed similar effects of irradiation on cytokinin activity in tomatoes.

## CONCLUSION

It seems that the gorwth rate directly relates with the cytokinins content, and irradiation treatment by direct and indirect hit mechanisms results in the destruction of this growth hormone and ultimately in the inhibition of growth in mushrooms' stipe. Further work is necessary to have a clear idea of the situation.

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