

Studies on the Effect of Different Insecticidal Applications on Quality, Nutritional Parameters and Total Antioxidant Capacity of Brinjal (*Solanum melongena* L.)

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Abstract. Brinjal (*Solanum melongena* L.) suffers from the attack of various pests throughout its different stages of the crop growth. Different insecticides are used to control these pest infestations. The application of these insecticides on brinjal plants may have an effect on their quality and nutritional parameters of fruit samples. In the present study effect of three different insecticides viz. Triazophos 40% E.C. Carbaryl 50% W.D.P, Cypermethrin 10% E.C. and Azadiractin 10,000 ppm formulation belonging to different groups namely organophosphate, carbamate, synthetic pyrethroids and botanical insecticide on brinjal fruit quality, nutritional parameters and total antioxidant capacity (TAC) were assessed in field conditions. The results showed that different insecticides at field application dose showed minute insignificant variation in fruit nutritional and total antioxidant activity parameters in comparison to the control samples. The fruit quality parameters also showed minute variation in comparison to the control, except in the case of Triazophos treated samples which showed significant variation ($p < 0.05$) in total soluble sugar content and crude protein.

Introduction

The nutritional features and antioxidant activity of fruits and vegetables are highly beneficial to health as they protect against cellular level oxidation reactions. The beneficial effect of fruits and vegetables are mostly dependent on quantity of numerous antioxidants [1-5]. The fruit quality parameters considered are soluble solids content, titratable acidity etc. while the nutritional parameters are total phenolic content and flavonoid content and Total Antioxidant Capacity (TAC) in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, reducing power and hydroxyl radical scavenging activity. The oblong-fruited brinjal cultivars are rich in total soluble sugars, while free reducing sugars, anthocyanins, phenols, glycoalkaloids content were higher in long fruited cultivators. Cao et al. [6] reported that brinjal has high oxygen radical absorbance capacity due to its phenolic content. Brinjal, is cultivated in India, as an important cash crop by farmers. The Indian scenario of cultivation of brinjal depicts that brinjal is cultivated over an area of more than 5,00,000 ha [7] and henceforth is a key source of cash for many farmers [8]. The average yield of brinjal in India has been reported to be around 200 to 350 quintals per ha [7]. Apart from the attack of various insect pest throughout the cropping season of brinjal, the biggest threat to brinjal cultivation in India is the widespread infestation of brinjal or eggplant by eggplant shoot and fruit borer (ESFB), which can damage 95% of the crop, during the rainy season [9]. To protect their crop, farmers have been

relying on insecticides, because alternative control measures have not been well developed [10]. The use of these pesticides on brinjal plants may have an effect on their quality and nutritional parameters. Pesticides have been earlier reported to affect the content of acids, sugars, and possibly other constituents, that might affect flavor [11, 12]. Tichneor et al. [13] studied the effect of 21 insecticides and five fungicides on strawberry. They found no off flavors in the frozen fruit that could be related to pesticide treatment. Murphy et al. [14] reported that thiodan has no effect on strawberry quality. Sweeney et al. [15] studied the effect of chlordan, demeton, captan and ferbam on quality of strawberry at their recommended dose of application. They reported little detectable effect on compositional factors, including sugars and acids, or on color, texture and flavor of fruit. They also reported no relationship between residue levels and quality of strawberries. Habiba et al. [16] reported that profenofos increased the percent protein content in potatoes. It is also reported that pirimiphos-methyl residues showed adverse effect on the content of total soluble sugars in tomato fruit and broad bean seeds [17]. Bertin et al. [18] reported non-significant reduction in titratable acidity content in carbaryl treated fruit samples. Radwan et al. [19] reported that residues of profenofos and pirimiphos-methyl, increased ascorbic acid content and decreased total soluble sugars, percent acidity and total suspended solids (TSS) content of brinjal/eggplant fruit. The present endeavour was therefore initiated to study the effect of four different insecticides application on quality, nutritional parameters and total antioxidant capacity of brinjal fruit samples collected from field conditions.

Materials and Methods

The experiment was in rabi season (November-March) of 2009-10 at Experimental Farm of University of Calcutta, Baruipur, (22°22'44.20" N 86°26'08.55" E) South 24 Parganas, West Bengal, India, in a Randomized Block Design (RBD) with 20 plots (measuring $4 \times 3 \text{ m}^2$), involving four different insecticidal treatments and control with each application plot having four replications. Brinjal variety (var. Muktakeshi) was used in the experiment.

The insecticides chosen for the study were purchased from local agro market and applied to the crops at recommended doses of field application. The treatment/dose details were Triazophos (3-(o,o-diethyl)-1-phenyl thiophosphoryl- 1,2,4-triazol) 40% EC (300 g.a.i/ha), Cypermethrin (RS)-alpha-cyano-3-phenoxybenzyl(1RS)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate) 10% EC (60 g.a.i/ha), Carbaryl (1- naphthalenyl methylcarbamate) 50 % WDP (1875 g.a.i/ha) and Azadirachtin 10000 ppm (5 g.a.i/ha). The conversion of the field application rate was made accordingly to the area of the plots ($4 \times 3 \text{ m}^2$). Insecticidal sprays were applied using a pressurized knapsack sprayer fitted with a single-cone nozzle at the rate of 500 L/ha. The spray dose was also calculated taking in consideration the area of the plots. The control plots received water treatment only. The insecticides were applied at an interval of 15 days following the pest attack, up to a threshold level to harvesting. In order to evaluate the effect of different insecticidal applications on brinjal fruit, analytical determinations of brinjal fruit quality, nutritional and total antioxidant parameters were made on both insecticide treated and untreated fruits. Fully mature brinjal fruits were picked 15 days after last application of insecticides. All the brinjal fruits used in the study were approximately of the same size, the same degree of maturity and not infested by pests.

Fruit quality parameters

Total soluble solids (TSS)

Brinjal fruit samples were taken for juice extraction and total soluble solids of the juice was determined by using a hand refractometer [20]. TSS was expressed in °Brix.

Titratable acidity (TA)

The juice from brinjal fruit samples was extracted from 5 g of brinjal fruit sample and filtered through muslin cloth. The volume was made up to 25 ml with distilled water. The mixture was then

titrated against standard NaOH, using phenolphthalein as an indicator. The appearance of light pink color was recorded at the end point. The result was expressed in terms of per cent acidity of the fruit [20].

Total soluble sugars

Total soluble sugars were measured following the method of Dubois et al. [21] with some The amount of total soluble sugars was calculated in mg 100g⁻¹ DW (Dry weight).

Crude protein

The crude protein was determined using micro Kjeldahl method as described in AOAC [22] with certain modifications. The crude protein content of the brinjal fruit samples was estimated by multiplying the percentage of nitrogen content by a factor of 6.25.

Fruit Nutritional parameters

The brinjal fruit samples collected from both insecticide treated and control field were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained, and dried in a hot air oven at 50°C. The dried fruits were finely powdered. The dried powder was extracted in 100 ml of (80:20) methanol and distilled water in a shaker for 8 hrs. The extract was decanted and extraction was repeated with fresh 80:20 methanol-water until the extract was colorless. The extracts were pooled and evaporated in a rotary evaporator below 40°C till the extract was free of the solvent.

Total polyphenol content

Total polyphenol content of brinjal fruit extract was determined by a method described by Mattheus [23] with certain modifications. The concentration was calculated using gallic acid as a standard, and the results were expressed as gallic acid equivalents (mg GAE) g⁻¹ extract.

Total flavonoid content

The total flavonoid content was determined by using the method of Lamien et al. [24] with certain modifications. Total flavonoid content was expressed as mg of quercetin equivalents (QAE) g⁻¹ extract.

Ascorbic acid content

Ascorbic acid content of brinjal fruit samples was determined according to 2, 6-dichlorophenolindophenol dye method [25]. Results were expressed as mg100g⁻¹ FW (Fresh weight).

Total antioxidant activity (TAC)

DPPH Assay

The free radical scavenging activity of methanol extracts of brinjal was measured by diphenyl-β-α-picryl-hydrazyl (DPPH) using the method described by Oktay et al. [26]. 0.1 mmol l⁻¹ solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of brinjal fruit extracts solution at concentrations of 50 µg. After 30 min absorbance (A) was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as inhibition percentage and was calculated using the formula.

$$\text{Radical Scavenging Activity (\%)} = \frac{(\text{Control A} - \text{Sample A})}{\text{Control A}} \times 100$$

Reducing power

The reducing power of brinjal fruit was determined according to the method of Oyaizu [27]. Brinjal fruit extract (50µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide K₃Fe(CN)₆ (2.5 ml, 1%). The mixture was incubated at

50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the methods described by Singh et al. [28]. The absorbance of the yellow colour formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging activity was calculated by the following formula,

$$\text{Hydroxyl Radical Scavenging Activity (\%)} = 1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100$$

Statistical analysis

Treatments were replicated three times in a completely randomized design. Analysis of variance (ANOVA) followed by post-hoc Test Duncan's multiple range test (DMRT) were applied to evaluate homogeneity or differences between the treatments using SPSS (Version 21.0) statistical software package. Differences obtained at a level of $P < 0.05$ were considered significant.

Results and Discussion

The results showing the effect of insecticidal applications on brinjal fruit quality are represented in Table-1. The result shows that the change in total TSS content of all the insecticide treated samples was found to be not statistically significantly different ($p < 0.05$), in comparison to the control. The maximum TSS content of 3.21⁰Brix was found in azadirachtin and cypermethrin treated sample, while minimum of 3.08⁰Brix was found in case of triazophos treated samples. The total soluble sugar content was significantly ($p < 0.05$) reduced in triazophos treated samples, in comparison to the control. Similar results were obtained by Radwan et al. [19] who reported that residues of profenofos, an organophosphate insecticide reduced the total soluble sugar content of eggplant fruit. Azadirachtin and cypermethrin treated fruit samples showed a non-significant increase, when compared to the control samples, while carbaryl treated fruit samples showed a decrease in the total soluble sugar content, but the decrease was found to be non-significant. Little [29] reported that sugars constitute 98.8% of the TSS content of fruits. It is noteworthy to mention here that the TSS content of the only triazophos treated fruit samples were found to be less than the control samples, since the total soluble sugar content in triazophos treated fruits was found to significantly ($p < 0.05$) less than the control. Interestingly, in all the other insecticides treated fruit samples though the increase or decrease in the TSS content of the fruit are found to be non-significant, but the increase or decrease in the TSS content in fruit samples followed the same trend, as in the case of total soluble sugars. The titratable acidity of fruits of azadirachtin and cypermethrin treated samples were found to be highest (i.e. 0.42%) amongst the treatments, but in comparison to control the increase in titratable acidity was non-significant. Carbaryl and triazophos treated fruit samples showed lesser titratable acidity content than the control. The more pronounce decrease in titratable acidity in triazophos treated fruit samples could be linked up to TSS and sugar content. Capocasa et al. [30] have reported significant correlation between TSS content and titratable acidity content in strawberries. It has also been reported that organic acids are produced in the fruit itself from stored carbohydrates [31]. The reduction in titratable acidity content in carbaryl treated samples was not significant, as in other cases. Teskey and Kung [32] reported that the carbaryl application in apple cultivation also did not influence the level of acids in apples. The crude protein content of azadirachtin, cypermethrin and carbaryl treated fruit samples was found to be non-significant in comparison to the control. However, in case of triazophos treated fruit samples it was found to be significantly ($p < 0.05$) different.

Table 1. Effect of different insecticidal treatments on brinjal fruit quality parameters.

Treatments	Fruit quality parameters			
	TSS (in ° Brix)	Titrateable acidity (% acidity)	Total soluble sugars (mg/100g D.W.*)	Crude Protein (% protein)
<i>Control</i>	3.17 ^{a*}	0.41 ^a	11.79 ^a	1.52 ^a
<i>Azadiractin 10000 ppm</i>	3.21 ^a	0.42 ^a	11.82 ^a	1.54 ^a
<i>Triazophos 40% E.C</i>	3.08 ^a	0.40 ^a	11.39 ^b	1.59 ^b
<i>Carbaryl 50% W.D.P</i>	3.19 ^a	0.40 ^a	11.72 ^a	1.53 ^a
<i>Cypermethrin 10% E.C</i>	3.21 ^a	0.42 ^a	11.82 ^a	1.53 ^a

Data are mean values of three replicates; *Means within a column followed by the same alphabetical letters are not significantly different at $p \leq 0.05$ by DMRT. *D.W. = Dry Weight.

The results showing the effect of different insecticidal applications on brinjal fruit nutritional and total antioxidant capacity parameters are represented in Table 2-3. The result shows that the total polyphenol content of the brinjal fruit samples collected from different insecticidal treatment plots were found to be statistically at par with the control at 5% level of significance. Maximum polyphenol content was found in azadirachtin treated fruit sample (i.e. 3.7 mg GAE g⁻¹ extract), while minimum was obtained in triazophos treated samples (i.e. 3.3 mg GAE g⁻¹ extract). Van Acker et al. [33] have reported that phenols and polyphenolic compounds, such as flavonoids found widely in food products derived from plant sources possess significant antioxidant activities. The flavonoid content also showed similar trend, in increase or decrease, as that of polyphenolic content. The change in flavonoid content in the insecticide treated samples were also non-significant at 5% level of significance in comparison to control. Maximum flavonoid content was found in azadirachtin treated fruit samples (i.e. 0.24 mg QAE g⁻¹ extract) which accounted for 20.83 percent increase over control samples. Though there is no significant ($p < 0.05$) variation in ascorbic acid content within the treatments, ascorbic acid (vitamin C) content was also found to be maximum in the azadirachtin treated samples (i.e. 5.11 mg 100g⁻¹ F.W) while the minimum ascorbic acid content of fruits was found in triazophos treated samples (i.e. 5.01 mg 100g⁻¹ F.W).

Table 2. Effect of different insecticidal treatments on brinjal fruit nutritional parameters.

Treatments	Fruit nutritional parameters		
	Total Polyphenol content (**mg GAE g ⁻¹ extract)	Total flavonoid content (#mg QAE g ⁻¹ extract)	Ascorbic acid content (mg 100g ⁻¹ F.W.*)
<i>Control</i>	3.4 ^a	0.20 ^a	5.03 ^a
<i>Azadiractin 10000 ppm</i>	3.7 ^a	0.24 ^a	5.11 ^a
<i>Triazophos 40% E.C</i>	3.3 ^a	0.18 ^a	5.01 ^a
<i>Carbaryl 50% W.D.P</i>	3.4 ^a	0.19 ^a	5.03 ^a
<i>Cypermethrin 10% E.C</i>	3.6 ^a	0.22 ^a	5.04 ^a

Data are mean values of three replicates; *Means within a column followed same alphabetical letters are not significantly different at $p \leq 0.05$ by DMRT. *F.W. = Fresh Weight. ** mg GAE/g extract = mg of gallic acid equivalents / g extract,

#mg QAE/g extract=mg of quercetin equivalents/ g extract.

Table 3. Effect of different insecticidal treatments on brinjal fruit total antioxidant activity.

Treatments	Total antioxidant activity(TAC)		
	DPPH assay % Radical Scavenging Activity	% Hydroxyl Radical Scavenging activity	Reducing power
<i>Control</i>	20.6 ^{a*}	24.8 ^a	0.887 ^a
<i>Azadiractin 10000 ppm</i>	21.5 ^a	25.3 ^a	0.891 ^a
<i>Triazophos 40% E.C</i>	20.5 ^a	24.7 ^a	0.886 ^a
<i>Carbaryl 50% W.D.P</i>	20.9 ^a	24.9 ^a	0.888 ^a
<i>Cypermethrin 10% E.C</i>	21.2 ^a	25.0 ^a	0.889 ^a

Data are mean values of three replicates; *Means within a column followed by the same alphabetical letters are not significantly different at $p \leq 0.05$ by DMRT.

Reports existing in literature shows a high correlation between phenolic content and antioxidant activity of various fruit and vegetables [34-40]. Flavonols such as quercetin and flavones in plant materials are also closely associated with their antioxidant function mainly due to their redox properties employed by various possible mechanisms including free-radical scavenging activity, transition-metal-chelating activity, and/or singlet oxygen- quenching capacity [41-42]. Shyamala et al. [43] observed a high positive correlation ($r = 0.88$) between the polyphenol content and hydroxyl radical scavenging activity. Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant is associated with the development of reducing power [44]. In the present study, hydroxyl radical scavenging and reducing power of brinjal fruit extracts at concentration of $50 \mu\text{g ml}^{-1}$ in case of all the treatments and the control were statistically par at 5% level of significance as determined by Duncan's Multiple range test (DMRT). Studies on the fruits and vegetables have revealed a linear correlation between the DPPH scavenging activity and polyphenol content [45, 46]. The free radical scavenging activity of brinjal fruit extracts as measured by DPPH assay also showed no significant ($p < 0.05$) variation between the treatments and the control. An interesting observation here is that though in carbaryl treated samples, the total polyphenolic content was same and the flavinoid content was less than the control samples, yet the free radical scavenging activity and hydroxyl radical scavenging activity were found to be marginally increased in carbaryl treated samples than control samples. This may because of the fact the ascorbic acid (vitamin C) content of carbaryl treated fruits were slightly more than the control samples. It has reported that the total antioxidant activity in strawberries mainly depends on vitamin C content along with polyphenols, flavonoid and anthocyanin [47, 48]. The results therefore reveals that samples with more ascorbic acid (vitamin C), polyphenolic or flavonoid content show more antioxidant capacity, but the variation in nutritional parameters and total antioxidant activity of brinjal fruit samples from insecticide treated plots were found to be nonsignificant.

Conclusion

Therefore, the study reveals that following insecticidal application at field application dose on brinjal, showed minor changes in fruit quality, nutritional parameters and total antioxidant capacity of brinjal fruit samples and in most of the cases the changes were found to be nonsignificant amongst the treatments. However, in the case of triazophos treated samples, a significant variation in total soluble sugar content and crude protein were obtained.

Conflict of Interest

The authors declare that there is no conflict of interest.

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