Original Article

IMPACT OF THE PESTICIDES MONOCROTOPHOS AND QUINALPHOS ON THE BIOCHEMICAL CONTENTS OF RED AMARANTH UNDER ARBUSCULAR MYCORRHIZAL FUNGUS INOCULATION

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Abstract
The present work deals with the impact of Monocrotophos and Quinalphos on the biochemical contents of Red Amaranth under AM fungus inoculation. The Red Amaranth plants are grown in pots in a split plot design with pesticides levels as main treatments (Recommended level (0.5), Below recommended level(0.1) and Above recommended level(1.5) ppm) and AM fungus as sub treatments (AM- uninoculated and AM+ inoculated). The experiments were replicated seven times. The Red Amaranth plants are raised in pots. The AM fungus (Glomus fasciculatum) were mixed with the sand and applied to the pot soil (10 kg /acre). The two pesticides Monocrotophos and Quinalphos were sprayed on 5th day at three different levels. Pots were irrigated as and when necessary. The plant samples were analyzed at three different intervals (10, 20 and 30 DAS).

The results indicated that the pesticides (Monocrotophos and Quinalphos) application, at the three rates (Recommended level, Below recommended level and Above recommended level) caused reduction in various biochemical contents such as the chlorophylls, protein, sugars, starch and amino acid when applied without AM fungus inoculation. Application of pesticides (Monocrotophos and Quinalphos) at recommended level 0.5 ppm along with AM fungus inoculation only increased the biochemical contents of the Red Amaranth.

Key words: Red Amaranth – pesticides – monocrotophos–quinalphos–AM fungus –biochemical contents.

1. INTRODUCTION

Red Amaranth is a tropical plant and performs well under tropical conditions in the world. India is a tropical country with red amaranth cultivation from times immemorial. Amaranthus shows a wide variety of morphological diversity among and even within certain species. Amaranthus is a common leaf vegetable throughout the tropics and in many warm temperate regions. It is very popular in India. They are a very good source of vitamins including vitamin A, vitamin K, vitamin B6, vitamin C, riboflavin and folate and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. Because of their valuable nutritional status, Amaranthus is being cultivated enormously.

Continuous use of chemical inputs such as pesticides has in damage to the environment, caused human ill-health, negatively impacted on agricultural production and reduced agricultural sustainability [1]. Fauna and flora have been adversely affected. Numerous short- and long–term human health effects have been recorded [2]. Human deaths are not uncommon. The decimation of beneficial agricultural predators of pest has led to the proliferation of several pests and diseases [1]. Despite all these impacts and costs, farmers continue to use pesticides in most countries at an increasing rate, while biological methods of pest control have become limited.

Pesticides are essential for controlling plant pests, and accordingly, improve the productivity of major crops including vegetables and legumes. Pesticides applied to leguminous crops constitute a potential hazard to growth, nodulation and nitrogen accumulation [3]. Sreenivasa and Bagyaraj reported that pesticides were toxic to AM fungus.
even at recommended levels [4]. Menge reported that systemic fungicides have a more harmful effect on AM fungi than non-systemic fungicides [5]. The effects of pesticides application on symbiosis in temperate and tropical soils has been studied extensively [6].

Among the various pesticides used in India, 40% of all the pesticides used belong to organochlorine class of chemical pesticides [7,8]. Monocrotophos, phorate, phosphomidon, methyl parathion and dimethoate are some of the highly hazardous pesticides that are continuously and indiscriminately used in India. The higher consumption of insecticides is partially due to warm humid and tropical climate which provides favorable breeding environment for insects coupled with shorter life cycle and higher hatching rate. Organophosphorus compounds have over taken organochloride compounds as the most used insecticides in the recent decades [9].

Application of pesticides for improving crop productivity has become necessary in the present day agricultural practices, results in entry of these chemicals into soil and water ecosystems. Amongst agricultural practices, the widespread use of plant protection products represents a potential threat to soil organisms [10], including diazotrophs resulting in either stimulatory or inhibitory effect [11, 12]. Many pesticides tested adversely affect the mycorrizal symbiosis [13] while others do not appear to damage mycorrhizal fungus. Under certain conditions, some pesticides may even increase mycorrhizal colonization and development [14]. Most of the pesticides, being xenobiotic, are degraded by very few microorganisms [15], which developed the tendency to degrade them while they be toxic for others. Extensive studies on the effect of some commonly used pesticides on the soil have been well worked out on chemotrophic bacteria and cyanobacteria [16]. The effect of some of the pesticides on beneficial microorganisms-Azospirillum [17], Rhizobium [18,19], Bradyrhizobium [20], cyanobacteria [16] Pseudomonads diazotrophic purple nonsulphur bacteria [11], and Azotobacter [21] have been well studied. For the enhacement of crop growth and to maximize the yield of red amaranth, various formulations of pesticides are used but their impacts on the biochemical contents remains uninvestigated. So the present study was carried out to know the impact of the pesticides, Monocrotophos and Quinalphos on the biochemicals (aminoacid, protein and sugars) contents of Red Amaranth under AM fungus inoculation.

2. MATERIALS AND METHODS

2.1. Pot culture

The present research work has been carried out in the Botanical garden of Annamalai University to find out the impact of the pesticides, Monocrotophos and Quinalphos on the biochemicals contents of Red Amaranth under AM fungus inoculation. Seeds of Red Amaranth were collected from Tamil Nadu Agricultural Research Institute, Palure, Cuddalore. The AM fungal species (Glomus fasciculatum) were collected from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Monocrotophos and quinilphos were collected from Local Agro Centre, Chidambaram, Tamil Nadu. For experiment purpose, AM fungus were mixed with sand (10 kg/acre) and applied to the pot culture. The pesticides were sprayed on 5th day on the field for 0.5, 0.10 and 1.5 ppm concentration. The experiment is a split plot design with pesticides levels as main treatments (Recommended level (0.5), Below recommended level (0.1) and Above recommended level(1.5) ppm) and AM fungus as sub treatments (AM- uninoculated and AM+ inoculated) as sub treatments. The experiments were replicated seven times. The biochemical contents such as sugars, amino acids and protein were estimated in the 10, 20, 30th days old seedlings grown in the pots.

2.2. Estimation of total sugar

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80% ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved and the content was made up to 20 ml and the extract was used for the estimation of total sugar by using the methods of Nelson [22].

2.3. Estimation of amino acids

Five hundred mg of plant materials were weighed and macerated with a pestle and mortar with 10 ml of 80% ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. The extract was used for the estimation of amino acids followed by the methods of Moore and Stein [23].

2.4. Estimation of protein

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 20% trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml with 0.1 N NaOH. This extract was used for the estimation of protein following the methods of Lowry et al. [24].

3. RESULTS

3.1. Total sugar

The effect of pesticides and AM fungus on total sugar content of red amaranth at various stages of its growth are presented in Table- 1. The higher total sugar content (2.140, 3.410 and 3.815) was recorded in AM fungus inoculation with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower total sugar content (0.418, 0.630 and 1.026) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS. The higher total sugar content (2.340, 2.840 and 3.630) was recorded in AM fungus with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower total sugar content (0.452, 0.663, and 1.127) was recorded in...
Table 1. Effect of pesticides on total sugar content (mg/g fr. wt.) of red amaranth under AM inoculation

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>Monocrotophos</th>
<th>Quinalphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after sowing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>AM (-)</td>
<td>0.617 ± 0.030</td>
<td>0.917 ± 0.045</td>
</tr>
<tr>
<td>AM (+)</td>
<td>1.179 ± 0.058</td>
<td>2.140 ± 0.107</td>
</tr>
<tr>
<td>BRL (RL)</td>
<td>0.733 ± 0.036</td>
<td>1.843 ± 0.092</td>
</tr>
<tr>
<td>ARL</td>
<td>0.418 ± 0.020</td>
<td>0.843 ± 0.042</td>
</tr>
</tbody>
</table>

Values are mean of seven replications ± Standard deviation, (-): Uninoculated; (+): Inoculated.
RL: Recommended Level, BRL: Below Recommended Level, ARL: Above Recommended Level.

Table 2. Effect of pesticides on amino acid content (mg/g fr. wt.) of red amaranth under AM inoculation

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>Monocrotophos</th>
<th>Quinalphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after sowing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>AM (-)</td>
<td>0.318 ± 0.015</td>
<td>1.841 ± 0.092</td>
</tr>
<tr>
<td>AM (+)</td>
<td>0.234 ± 0.011</td>
<td>0.974 ± 0.048</td>
</tr>
<tr>
<td>BRL</td>
<td>0.186 ± 0.009</td>
<td>0.631 ± 0.031</td>
</tr>
<tr>
<td>ARL</td>
<td>0.374 ± 0.018</td>
<td>2.130 ± 0.106</td>
</tr>
</tbody>
</table>

Values are mean of seven replications ± Standard deviation, (-): Uninoculated; (+): Inoculated.
RL: Recommended Level, BRL: Below Recommended Level, ARL: Above Recommended Level.

above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

3.2. Amino acid

The effect of pesticides and AM fungus on amino acid content of red amaranth at various stages of its growth are presented in Table 2. The higher amino acid content (0.974, 1.314 and 1.933) was recorded in AM fungus inoculation with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower amino acid content (0.374, 0.504 and 0.586) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS. The higher amino acid content...
content (0.986, 1.687 and 2.113) was recorded in AM fungus inoculation with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower amino acid content (0.400, 0.684, and 0.710) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

3.3. Protein

The effect of pesticides and AM fungus on protein content of red amaranth at various stages of its growth are presented in Table-3. The higher protein content (0.963, 1.613 and 1.993) was recorded in AM fungus inoculation with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower protein content (0.115, 0.380, and 0.401) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS. The higher protein content (0.515, 1.010, and 1.615) was recorded in AM fungus inoculation with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower protein content (0.088, 0.263, and 0.318) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

4. DISCUSSION

Biochemical parameters such as total sugars, amino acid and protein content of red amaranth are higher in recommended level of pesticide application supplemented with AM fungi. The lower results were recorded in above recommended level of pesticide application without AM fungus inoculation. In case of sampling days all the parameters were higher in recommended level of pesticide with AM fungi application at 30 DAS plants.

The reduction in biochemical contents of the plants tested with pesticide application is mainly due to the harmful effects of these chemicals on root colonization with AM fungi. This can be comparable with the work of Abd Alla et al. [25] in their study, single application of endosulfan at recommended rates does not inhibit plant growth and AM development while two repeated applications at these concentration adversely affects all the parameters except plant height.

The biochemical constituents like total sugars, amino acids and proteins were increased significantly in tetrapartiate associations in leguminous plants. Green house studies conducted by Cabello et al., showed that the dual inoculation of AM fungus Glomus mosseae and a phosphorus solubilizing microorganism Penicillium thomii in Mentha piperita with or without rock phosphate, showed a positive effect of these microbes on host plant's growth when compared to control [26]. Dual inoculation of leguminous plants with Rhizobium and AM fungus was found to enhance chlorophyll content and photosynthetic rates in Cyamopsis sp. Rice bean (Vigna umbellata) inoculated with G. fasciculatum and Rhizobium sp. in a P deficient soil significantly increased AM colonization, nodulation and yield of plants.

In an attempt to show movement of donor – plant organic compounds to the recipient, Robinson and Baydorfer applied N as labeled ammonia ($^{15}$NH$_4$NO$_3$) to the cocklebur leaves to encourage its rapid assimilation into amino acids.

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**Table 3. Effect of pesticides on protein content (mg/g fr. wt.) of red amaranth under AM inoculation**

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>Monocrotophos</th>
<th>Quinalphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>AM (-)</td>
<td>0.218 ± 0.010</td>
<td>0.415 ± 0.020</td>
</tr>
<tr>
<td>AM (+)</td>
<td>0.218 ± 0.010</td>
<td>0.415 ± 0.020</td>
</tr>
<tr>
<td>RL</td>
<td>0.346 ± 0.017</td>
<td>0.983 ± 0.049</td>
</tr>
<tr>
<td>BRL</td>
<td>0.346 ± 0.017</td>
<td>0.983 ± 0.049</td>
</tr>
<tr>
<td>ARL</td>
<td>0.115 ± 0.005</td>
<td>0.318 ± 0.015</td>
</tr>
</tbody>
</table>

Values are mean of seven replications ± Standard deviation, (–): Uninoculated; (+): Inoculated.

RL: Recommended Level, BRL: Below Recommended Level, ARL: Above Recommended Level.
It also suggests that transfer of organic compounds from endophyte to host plant does take place, perhaps in the form of amino acids [28]. The increased amino acid in the amaranthus plants inoculated with AM fungus and recommended level of pesticides application might be the cause for the transfer of amino acid from AM fungus to the amaranthus plants as reported by Cliquet and Stewart [28].

Pesticides also have deleterious impacts on algae by influencing their growth, photosynthesis, nitrogen fixation, biochemical composition, metabolic activities, etc [29]. Recently, Frischen et al., investigated the effect of a fungicide, an insecticide, and biopesticide on Tolypothrix scytoneoides [30]. They reported that the rates of photosynthetic oxygen evolution decreased but rates of respiratory oxygen consumption were increased in cells exposed to these pesticides. The chlorophyll or protein contents in algae cells were reduced with increasing concentration of bensulfuron methyl, exhibiting the good concentration–effect relationship [31]. Kinney et al., revealed that surfactant alkyl benzensulfonate (LAS) and the heavy metals Hg²⁺, Al³⁺ and Cu²⁺ and / or their mixture (all are used as pesticides) markedly altered (≥50%) acid phosphate activity of Pseudokirchneriella subcapitata[32]. Here also the application of pesticides monocrrotofoths and quinalphos at above recommended level without AM fungus inoculation decreased the protein content of amaranthus plants.

Soil microorganisms have the ability to carry out biochemical transformations of various elements like nitrogen (N), phosphorus (P), sulfur(S) and carbon (C). Pesticides may directly or indirectly affect the vital biochemical reactions such as mineralization of organic matter, nitrogen fixation, nitrification, denitrification and ammonification by activating/deactivating specific soil microorganism and/or enzymes [33, 34]. Information on possible effects of pesticides on all biochemical processes is sparse; however, a description of pesticides effects on soil biochemical reactions is summarized [35]. It is difficult to quantify the net impact of pesticides on biochemical reactions in soil due to greater soil resilience, nature and concentration of pesticide, its activity and metabolism in soil and production of metabolites. But in most cases, application of pesticides can disturb microbial biochemical equilibrium and cycling of biological elements. The effect of pesticides on soil enzymes particularly extracellular enzymes are not clear due to their multidimensional behavior in complex soil medium and the greater complexity of soil microbial and biochemical interactions[36]. Here the decreased biochemicals contents of red amaranth plants at below recommended level and above recommended level of pesticides application without AM fungus inoculation and increased biochemical contents of the red amaranth plants at recommended level of pesticides application with AM fungus inoculation might be to some extent due to the above said facts.

The increased biochemical contents such as total sugar, amino acids and proteins contents of Red Amaranth in recommended level of pesticides application supplemented with AM fungi might be due to active involvement of AM fungi in enhancing growth by supplying some essential nutrients.

REFERENCES


8. FAO, Questionnaire on the state of implementation of the international code of conduct on the distribution and use of pesticides, (1994) Rome.


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