Impact of the pesticides moncrotophos and quinalphos on the nutrient contents of red amaranth under arbuscular mycorrhizal fungus inoculation

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Abstract
The present work deals with the impact of the pesticides, Monocrotophos and Quinalphos on the nutrients contents of Red Amaranth (Amaranthus tricolor L.) 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 30DAS). 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 nutrients contents such as nitrogen, phosphorus and potassium 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 nutrients contents of the Red Amaranth.

Keywords: Red Amaranth - Amaranthus tricolor – pesticides - moncrotophos-quinalphos- AM fungus - Glomus fasciculatum –Nutrients - nitrogen - phosphorus - potassium.

1. Introduction
Red Amaranth (Amaranthus tricolor L.) is a common leafy vegetable throughout the tropics and in many warm temperate regions. It is very popular in India. It contains very good source of vitamins including vitamin A, vitamin K, vitamin B6, vitamin C, riboflavin and foliate and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. Because of its valuable nutritional contents, farmers grow enormously amaranth plants. Red Amaranth (Amaranthus tricolor L.) plays an important role in nutrition among the leafy vegetables grown in India. The leafy amaranth is said to be the native of India [1-2]. Among the leafy types, Amaranthus tricolor L. is the most commonly cultivated species in India. It is cultivated all over the country in any season due to its adaptability to wide range of soil and climate. However, during winter its growth and development is slower than summer and rainy season [3]. Growth, yield and quality of red amaranth depend on nutrient availability in soil, which is related to the judicious application of manures and fertilizers. Nutrients may be applied through two sources viz. organic and inorganic sources. Increased use of inorganic fertilizers in crop production deteriorates soil health, causes health hazard and creates imbalance to environment by polluting air, water, soil etc. The continuous use of chemical fertilizers badly affects the texture and structure, reduces organic matter content and decreases microbial activities of soil. A good soil should have an organic matter content of more than 3%. But in Bangladesh, most soils have less than 1.5%, some soils have less than 1% organic matter [4]. In continuous cropping area, organic matter supply to the crop field through different manuring practices is made only to a minimum extent [5].

The agricultural production increased tremendously due to introduction of high-yielding varieties, use of agrochemicals and improved irrigation facilities [6]. However, there are several constraints for further increase in agricultural production. One of the limiting factors is the increased incidence of pests and disease. On the other hand, increase use of chlorinated non-degradable pesticides have residue in various living systems for prolonged periods of
their span and are presumably responsible for a variety of toxic symptoms [7]. Rekha et al.[8] reviewed the technology of application of pesticides in India and recommended future strategies for the rational use of pesticides and minimizing the problems related to health and environment due to inappropriate application of pesticides. So the present research work was carried out to know the impact of the two pesticides, Monocrotophos and Quinalphos on the nutrients contents of Red Amaranth under AM fungus inoculation.

2. Materials and methods
2.1. Plant materials and Cultivation
The present research work has been carried out in the Botanical garden of Annamalai University to find the impact of Monocrotophos and Quinalphos on the nutrient contents of Red Amaranth under AM fungus inoculation. Seeds of Red Amaranth were collected from Tamil Nadu Agricultural Research Institute, Palure, and Cuddalore. The AM fungal species (Gleomus fasciculatum) were collected from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, and Coimbatore. Monocrotophos and quinolophos were collected from local Agro Centre, Chidambaram, and Tamil Nadu. The Red Amaranth plants are raised in pots. The pesticides were sprayed on 5th day on the plants at three different (0.5, 0.1 and 1.5 ppm) concentrations. For experiment purpose, AM fungi were mixed with sand (10 kg/acre) and applied to the pot culture. 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). The experiments were replicated seven times. Ten seedlings were randomly selected on 10, 20 and 30th day from each treatment to analyse the nutrients contents. The seedlings were kept in a hot air oven at 80°C for 24 h. Then, the samples were kept in desiccators and powdered for nutrients analysis.

2.2. Nutrient Content Estimations
2.2.1. Estimation of nitrogen [9]
Hundred milligrams of dried materials were taken in the Kjeldahl flasks and 5 ml of a salicylic-sulphuric acid mixture (5 g salicylic acid in 100 ml concentrated sulphuric acid) was added. The flask was rotated to mix and allowed to stand for 30 minutes. Approximately 0.3g sodium thiosulphate was added and the resulting mixture heated gently until fumes appeared. Then 5 ml of concentrated sulphuric acid and approximately 0.1 g of catalyst mixture (Copper sulphate, potassium sulphate and selenium dioxide mixed in the ratio of 1:8:1) were added. Digestion was performed at low heat until frothing stopped and fumes of sulphuric acid freely evolved. After 5-10 minutes heat was increased, so that the acid boiled and condensed one third way up to neck of the flask. Digestion was continued for at least 3 hours, until the digest had become colourless. Upon completion of the digestion, the flask was cooled, and 20 ml of water was added. The flask was again cooled, the content was transferred into a 50 ml volumetric flask, and the volume was made up with distilled water. Distilled water was boiled in the flask and clips were kept closed and opened respectively when steam passed through the funnel and the funnel was washed twice with 1 ml of water each time. The ground glass stopper was replaced and 8 ml of 40% sodium hydroxide was added through the funnel. The lower end of the condenser was kept submerged into 5 ml of 2% boric acid and few drops of mixed indicator (6 ml of methyl red solution (0.16% in 95% alcohol) and 12 ml bromocresol green (0.04% in water) were mixed, and 6 ml of 95% alcohol was added to the mixture) contained in a 50 ml conical flask. When steam issued freely through the tube, the clip was closed and the ground glass stopper was lifted to allow sodium hydroxide to run into the digest. The stopper was immediately replaced and distillation continued until 30 ml of distillate was collected. After a few ml of liquid had distilled over, the end of the condenser was raised above the level of boric acid. Heating was stopped after distillation was completed, so that the liquid in the distillation chamber was automatically sucked into the jacket. A few ml of water was added through the funnel and the stopper was replaced. Then liquid in the jacket was allowed to run as waste by opening the clip. The apparatus was then ready for the next distillation. The whole distillate was titrated against standard 1/28 hydrochloric acid solution just until the pink color reappeared. Blank digestion, distillation and titration, were performed using all the reagents, without a plant sample. The percentage of total nitrogen was calculated by the following formula.

Percentage of nitrogen = (T-B) × 5 × N × 1.4/S

Where,
T = Sample titrated (ml)
B = Blank titrated (ml)
N = Normality of hydrochloric acid (1/28 = 0.0357142)
S = Weight of plant material (g)
Aliquot factor = 5

2.2.2. Estimation of phosphorus [10]
One gram of dried powdered plant material was digested with 10 ml of acid mixture (nitric acid 750 ml, sulphuric acid 150 ml and perchloric acid 300 ml). The digest was cooled and made up to 50 ml and filtered. One ml of the digest was mixed with 2 ml of 2N nitric acid and diluted to 8 ml. One ml of molybdovanadate reagent (25 g of ammonium molybdate in 500 ml of water, 1.25 g of ammonium vanadate in 500 ml of 1 N nitric acid, both were mixed in equal volume) was added, shaken, and the absorbance was measured at 420 nm in UV-Spectrophotometer after 20 min. Calibration curve was prepared using potassium dihydrogen phosphate as standard.

2.2.3. Estimation of Potassium [11]
Dried powdered plant material (0.5g) was digested in 100 ml Kjeldahl flask using 10 ml of concentrated nitric acid, 0.5 ml of 60% perchloric acid and 0.5 ml of sulphuric acid. The inorganic residue was cooled and diluted with 15 ml of distilled water and filtered through Whatmann No. 42 filter paper. The filtrate was made up to 50 ml with distilled water. The filtrate was used for potassium estimation by Flame photometer and standards were prepared with potassium chloride.
3. Results

3.1. Nitrogen

The effect of pesticides and AM fungi on nitrogen content of red amaranth at various stages of its growth is presented in Table-1. The higher nitrogen content (2.13, 2.86, and 3.14) was recorded in AM fungi with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower nitrogen content (0.48, 0.76, and 1.03) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS.

The higher nitrogen content (2.51, 2.78, and 3.68) was recorded in AM fungi with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower nitrogen content (0.40, 0.58, and 0.98) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

3.2. Phosphorus

The effect of pesticides and AM fungi on phosphorus content of red amaranth at various stages of its growth is presented in Table-2. The higher phosphorus content (3.76, 4.15, and 5.63) was recorded in AM fungi with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower phosphorus content (0.83, 0.97, and 1.10) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS.

The higher phosphorus content (3.89, 4.80, and 5.93) was recorded in AM fungi with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower phosphorus content (0.96, 1.10, and 1.28) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

3.3. Potassium

The effect of pesticides and AM fungi on potassium content of red amaranth at various stages of its growth is presented in Table-3. The higher potassium content (3.15, 3.73, and 4.00) was recorded in AM fungi with recommended level of monocrotophos application at 10, 20 and 30 DAS. Similarly the lower potassium content (1.00, 1.31, and 1.84) was recorded in above recommended level of monocrotophos application without AM fungus inoculation at 10, 20 and 30 DAS.

The higher potassium content (3.38, 4.00, and 4.28) was recorded in AM fungi with recommended level of quinalphos application at 10, 20 and 30 DAS. Similarly the lower potassium content (1.00, 1.16, and 1.19) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

<table>
<thead>
<tr>
<th>Table 1. Effect of pesticides on nitrogen content (mg/g dry wt.) of red amaranth under AM inoculation</th>
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<tbody>
<tr>
<td>Treatments (ppm)</td>
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<tr>
<td>Control</td>
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<tr>
<td>RL(0.5)</td>
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<td>BRL(0.1)</td>
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<td>ARL(1.5)</td>
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Values are mean of seven replications ± Standard deviation. (–): Uninoculated; (+): Inoculated

RL: Recommended Level, BRL: Below Recommended Level, ARL: Above Recommended Level

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<th>Table 2. Effect of pesticides on phosphorus content (mg/g dry wt.) of red amaranth under AM inoculation</th>
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<tr>
<td>Treatments (ppm)</td>
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<td>Control</td>
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<td>RL(0.5)</td>
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<td>ARL(1.5)</td>
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Values are mean of seven replications ± Standard deviation. (–): Uninoculated; (+): Inoculated

RL: Recommended Level, BRL: Below Recommended Level, ARL: Above Recommended Level
Table 3. Effect of pesticides on potassium content (mg/g dry wt.) of red amaranth under AM inoculation

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>Monocrotophos</th>
<th>Quinalphos</th>
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<tbody>
<tr>
<td></td>
<td>Days after sowing</td>
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<td></td>
<td>AM (-)</td>
<td>AM (+)</td>
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<tr>
<td>RL(0.5)</td>
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<tr>
<td>2.78</td>
<td>±0.139</td>
<td>3.15</td>
</tr>
<tr>
<td>1.96</td>
<td>±0.059</td>
<td>1.19</td>
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<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.15</td>
<td>±0.108</td>
<td>2.85</td>
</tr>
<tr>
<td>RL(0.1)</td>
<td></td>
<td></td>
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<tr>
<td>1.85</td>
<td>±0.092</td>
<td>2.10</td>
</tr>
<tr>
<td>1.00</td>
<td>±0.050</td>
<td>1.19</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

lower potassium content (0.93, 1.00, and 1.80) was recorded in above recommended level of quinalphos application without AM fungus inoculation at 10, 20 and 30 DAS.

4. Discussion
The nutrients (N, P and K) contents of Red Amaranth was higher in plants treated with recommended level of pesticides supplemented with AM fungi than the application of pesticides alone. All the parameters were higher in recommended level of pesticide with AM fungi application at 30 DAS plants. Among the pesticides all the values were higher in quinalphos than in monocrotophos. It may be due to the higher sensitivity of red amaranth plants towards the monocrotophos than the quinalphos. The decrease in N, P and K contents of the plants may be due to application of pesticide at above recommended level which affects the root colonization with AM fungi. This can be compared with the work of Abd-Alla et al. [12] who found that single application of endosulfan at recommended rates does not inhibit plant growth and VAM development while two repeated applications adversely affects all the parameters except plant height.

The nitrogen contents of Red Amaranth was higher in plants treated with recommended level of pesticides (monocrotophos and quinalphos) supplemented with AM fungi. In agreement with these results, Vejasdova et al. [13] have shown that mycorrhizal plants fix considerably more nitrogen than non-colonized ones. Li et al. [14] reported that the mycorrhizal infection improved nitrogen content in the shoots and seeds of nodulated plants than the non-nodulating ones. Dual inoculation of leguminous plants with Rhizobium and AM fungus was found to enhance chlorophyll content and photosynthetic rates in *Cyamopsis* sp. [15]. 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 [16]. Govindarajulu et al. [17] also reported that a significantly positive correlation exists between percent AM infection and nodule number in 60 days old greenhut. In our study the improved activity of AM fungus at recommended level of pesticides application supplemented with AM fungi is responsible for the increased nitrogen content of red amaranth plants.

The decrease in the nitrogen content of plant tissue reported here is not surprising and is mainly due to the side effects of pesticides on AM fungi. Abd-Alla and Omar [18] reported a similar reduction in nitrogen content, concomitant with a decrease in nodule number, in faba bean plants grown in herbicide-treated soil. The main source of plant nitrogen was N2 fixed in nodules as the N content of the soil was very low. The decrease in nodule number on pesticide-treated plants may explain the decrease in tissue N. Here the pesticides might exert a negative role on AM fungus which is responsible for the reduction in nitrogen contents of red amaranth under above recommended level of pesticides (monocrotophos and quinalphos) applications.

The phosphorus content of red amaranth plants was higher in AM fungi with recommended level of pesticides (monocrotophos and quinalphos) application. It is due to the enhanced supply of phosphorus by AM fungi. Yao et al.[19] reported that Arbuscular Mycorrhizal (AM) fungi and Rhizobacteria could interact synergically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition (Nitrogen and phosphorus bioavailability) and inhibition of fungal plant pathogens. Porcel et al. [20] reported that the dual inoculation with *Glomus intraradices* and *Bradyrhizobium* in *Acacia mangium* under aeroponic culture, showed increased growth rate than single or non-inoculated ones.

The role of mycorrhizal fungi in P uptake by plants is well documented and considered as the chief mechanism involved in plant-mycorrhiza symbiosis [21-23]. Mycorrhizas markedly increased the P uptake, growth and nodulation in clover at low and intermediate rates of applied phosphorus [24]. Arroca et al.[25] reported that a satisfactory nodulation was greatly dependent on the mycorrhizal symbiosis. *Rhizobium* and *Phosphobacteria* cultures improved plant growth, nodulation and mycorrhiza formation. The important benefit of AM association with leguminous plants is the absorption of phosphates from soil.
Phosphorus is an important macronutrient for leguminous plants due to its role in energy transfer during the process of nitrogen fixation. A good supply of phosphorus is essential for effective nodulation, which could be accomplished by AM association. Nodules are generally known to possess higher concentration of phosphorus than root tissues [26]. The requirement of P is high in legumes and therefore leguminous plants respond more to mycorrhizal infection than cereals, which indirectly enhances the biological nitrogen fixation through increased P availability especially in soils with low P content [27]. Legumes can form two types of associations with microorganisms. One with *Rhizobium* sp. involved in the fixation of atmospheric nitrogen and the other with fungi, that form vesicular Arbuscular Endomycorrhizas which is concerned with the uptake of phosphorus and other nutrients [24]. Inoculation of plants with VA mycorrhizal fungi can stimulate nodulation and nitrogen fixation by legumes [28].

The potassium content of red amaranth plants was higher in AM fungi with recommended level of pesticides (monocrotophos and quinalphos) application. It is due to the enhanced supply of potassium by AM fungi. Also, some authors demonstrated conclusively that plant colonization with AM fungi enhanced K uptake from the soil and improved the K status of colonized plants [29-30]. The application of pesticides (monocrotophos and quinalphos) at above recommended level lowered the potassium content of red amaranth plants. The negative effects of pesticides on P and K uptake by plants owing to the effects of these pesticides on root colonization with AM fungi, may be indirectly involved in decreasing nitrogen fixation and consequently, total plant nitrogen through the decrease in adequate supply of these elements to roots and nodules [31-32]. In accordance with these suggestions, Mosse et al. [33] reported that many tropical legumes nodulated in phosphate-deficient soils only when they were mycorrhizal. Also, Abd El-Maksoud et al. [34] and Ishac et al. [35] have shown that the colonization of legumes with AM fungi is an important perquisite for adequate yield of plant grown in calcareous soil. Enhancement of growth by AM fungi could possibly be due to phytohormonal production by these microorganisms that may be changed by the action of pesticides [36].

AM fungi may enhance N uptake, improve P and K nutrition and impair disease resistance in their host plants or adaptation to various environmental stresses. These agriculturally important symbiotic microorganisms play a remarkable role in macro nutrients (N, P, K) and micro elements acquisition [37]. The higher doses of pesticides (monocrotophos and quinalphos) application not only suppress the red amaranth growth and uptake of nutrients but also the AM fungus. Whereas the application of pesticides (monocrotophos and quinalphos) at recommended level with AM fungus inoculation increased the nutrients (N, P, and K) contents of red amaranth.

5. Conclusion

The nutrient contents (N, P and K) of red amaranth increased in the application of pesticides (monocrotophos and quinalphos) at recommended level with AM fungus (*Glomus fasciculatum*) inoculation in all the three sampling days. The nutrients contents of red amaranth plants were higher in the AM inoculated plants than the uninoculated plants. Application of pesticides at below recommended level and above recommended level with and without AM fungus inoculation causes reduction of nutrients contents of red amaranth plants.

References

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