

# Screening and Evaluation of the Effect of Exogenous Application of ABA and Propiconazole on the Antioxidant potential of *Mucuna pruriens* seed Extracts

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## ABSTRACT

The aim of our present study was the effect of soil drenching of propiconazole (PCZ) and Abscisic Acid (ABA) on vegetative growth, biochemical changes and antioxidant activities in seed methanolic extracts of *Mucuna pruriens* (MEMP). In this research we evaluated the morphological parameters like fresh and dry weight of plant, number of pods and antioxidant assay such as Total phenol, Total flavanoid, 1-diphenyl 2-picryl hydrazyl (DPPH), Superoxide radical scavenging activity, Metal chelating and Ferric ion Reducing antioxidant activity (FRAP). The report shows Number of pods, Number of seeds were increased while the plant treated with PCZ than the ABA as well as control. The increased content of total phenol and flavanoids observed in treated plants than control. On the other hand the highest Superoxide radical scavenging activity, Metal Chelating activity, Ferric ion reducing antioxidant was found in PCZ treated extracts followed by ABA treated extracts and control. Based on our results it seems that PCZ considerably increased the antioxidants content than the ABA and control.

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## INTRODUCTION

*Mucuna pruriens* commonly called cow-hedge is an important medicinal plant belongs to the family Fabaceae. It has potentially important nutritional value as a rich source of protein (23-35%) as reported (Brassini 2002). Its use as a food or feed has been limited by the presence of anti-nutritionals and toxic compounds. All part of plants are known to possess high medicinal value (Causis 1989, Warriar *et al.*, 1996) and useful photo chemicals (Morris 1999). Roots of *Mucuna* are used in ayurveda and indigenous medicines to relieve constipation, nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcer and fever. *M. pruriens* had great demand in the international market after finding the L-DOPA, which serve as potential drug as anti parkinsons and provide symptomatic relief (Nagashayana *et al.*, 2000). Triazole compounds are made up of ring structure containing three nitrogen atoms, Chlorophenyl and Carbon side chain (Fletcher *et al.*, 1986). Effectiveness of fungicide or plant growth regulator is determined by the stereo-

chemical configuration of the constituents of Carbon chain (Fletcher and Hafstra, 1988).

Certain triazole compounds interfere with gibberellins biosynthesis and function as plant growth regulators. The plant growth regulating properties of triazole were interfering with the isoprenoid pathway and thus modulating the balance of important hormones including GA, ABA and Cytokine. The ultimate effects of triazole are therefore dependent on the dynamic equilibrium of these hormones at a specific state of plant growth and development (Fletcher *et al.*, 2000). Triazole compound like Paclobutrazol, Uniconazole, Propiconazole and Hexaconazole have growth regulating properties. It induces many morphological and metabolic changes like reduction in shoot elongation, stimulation of rooting, inhibiting gibberellins biosynthesis. Increased chlorophylls content change in carbohydrate status and increased by cytokines synthesis (Davis *et al.*, 1986; Fletcher and Hofstra, 1988 Morab *et al.*, 2003). Propiconazole is a systematic fungicide with broad range specificity fungal pathogen (Basarab and Pitteritti, 1991, Looflar, Hayes 1992, Sancholle *et al.*, 1984). Triazole treated plants typically appeared dark greener appearance has been correlated with increased chlorophyll content (Fletcher *et al.*, 2000).

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Abscisic acid (ABA) a terpenoid phytohormone, is involved in the regulation of many aspects of plant growth and development including seed maturation processes (Wasilewska *et al.*, 2008). The present study was investigating the morphological, and antioxidant activities induced by the Propiconazole and ABA.

## MATERIALS AND METHODS

### Cultivation methods

The *M.pruriens* seeds were collected from Tamil Nadu Agriculture University, Coimbatore Tamil Nadu. The plant were raised at agricultural field at Nanjai makathu vazhkai village during the month of April to September 2011. In a randomized block design 3 replicates of *M.pruriens* seeds were sown in a well manured field with spacing of 1.0 m x 1.0 m, 40 plants in 0.01 acre. Recommended dose FYM (1:2:1) were applied before sowing.

### Triazole Treatment

In the preliminary experiments 10, 20, 30 and 40 mg L<sup>-1</sup> PCZ was used for the treatment to determine the optimal concentration of Propiconazole. Among the treatment 40 mg L<sup>-1</sup> showed significant changes in altering seed germination and growth of *M.pruriens*. Likewise ABA with different concentration of 10, 20, 30 and 40 µg/mL used to determined the optimal concentration for treatment.

Among the concentration the 40 µg/mL ABA brought significant changes in seed germination and growth. The initial treatment was given by the soaking seeds in 40 µg/mL PCZ and 40 µg/mL ABA for 24 hrs. then the treated seeds were sown in the prepared field. Further treatment with PCZ and ABA were given on 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup>, 120<sup>th</sup> and 150<sup>th</sup> days {180 days crop} up to initial flower settings.

The treatments were given by soil drenching method. The matured seeds collected from the control and treatment plants were dried in shade and powdered in a mechanical grinder.

The three powder such as those obtained from control and treatment plants (PCZ & ABA) was extracted separately by 1000 ml of methanol using a Soxhlet extractor for 72 h at temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatmann filter paper (No. 1) and then concentrated in vacuum and dried. The 3 extract namely MEMP as control, seed extract obtained from plants treated with propiconazole namely MEMP(PCZ) and ABA treated plants seed extract namely MEMP(ABA) were directly used in biochemical assay.

### Growth Parameters

#### Total fresh weight and dry weight

The randomly harvested plant samples were separated into shoot and leaves and fresh weight was recorded. Then, the material was dried in an oven at 60°C until constant dry weight was obtained and the dry weight was expressed in grams per plant.

### Total number of pods

The total number of pods were counted for each plant and expressed as numbers of pods per plant.

### Total number of seeds

The total number of seeds were counted for each plant and expressed as number of seeds per pods.

### Antioxidant assay

#### Total phenolic content (TPC)

Total phenolic contents of seed extracts were determined using Folin–Ciocalteu assay (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, 100 mg of methanolic extracts were individually dissolved in 10 ml of methanol. Then, 0.1ml of these solutions was mixed with 2.5 ml of 10-fold diluted Folin–Ciocalteu reagent, and 2.0 ml of 7.5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). After incubation at 40 °C for 30 min, the absorbance of the reaction mixtures were measured at 760 nm by using a spectrophotometer (Hitachi UV- Japan). Gallic acid was used as a standard and TPC of seed extracts were expressed in milligram gallic acid equivalents (mg GAE/g dry weight).

#### Total flavonoid content (TFC)

Total flavonoid content was determined by the aluminium calorimetric method (Quettier-Deleu *et al.*, 2000), using rutin as a standard. Briefly, the test samples were individually dissolved in DMSO. Then, the sample solution (150 mL) was mixed with 150 mL of 2% AlCl<sub>3</sub>. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm by using a spectrophotometer (Hitachi - UV-1700, Shimadzu, Kyoto, Japan). Three replicates were made for each test sample. The total flavonoid content was expressed as rutin equivalents in microgram per gram extract (µg RE/g extract).

#### DPPH scavenging activity

DPPH scavenging activity of seed extracts was determined according to the method described by Singh, Murthy, and Jayaprakasha (2002) with slight modifications. In brief, 0.1 ml seed extract at various concentrations were respectively added to 0.49 ml of methanol and 0.39 ml of DPPH methanolic solution (4 mg/100 ml). Then, the mixtures were vortexed vigorously and allowed to stand in the dark for 60 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer (Hitachi UV-1700, Shimadzu, Kyoto, Japan) at 515 nm. The sample concentration providing 50% of radical scavenging activity (IC<sub>50</sub>) was obtained through interpolation of linear regression analysis. The lower IC<sub>50</sub> indicates higher radical scavenging activity and vice versa. Ascorbic acid and α-tocopherol were used as standards.

#### Superoxide radical scavenging activity

Superoxide radical scavenging activity study was performed according to the method of Martinez *et al.* (2001).

Percentage radical scavenging activity (RSA) was calculated using the formula:  $RSA\% = \frac{OD \text{ of control} - (OD \text{ of sample} - OD \text{ of sample control})}{OD \text{ of control}}$ .

**Metal chelating activity**

The chelation of ferrous ions by the extract was estimated by the method of Dinis *et al.* (1994) with slight modification and compared with that of EDTA, BHT and that of ascorbic acid. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula: Percentage of chelation =  $\frac{Ac - As}{Ac} \times 100$  where ‘Ac’ is the absorbance of control, ‘As’ is the absorbance of sample.

**Ferric ion reducing antioxidant power (FRAP) assay**

The FRAP assay was carried out as described by Stratil and others (Stratil *et al.*, 2006) with slight modifications. The FRAP reagent was made fresh before each experiment. The FRAP reagent was prepared by mixing 38 mM sodium acetate anhydrous in distilled water pH 3.6, 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in distilled water and 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl in a proportion of 10:1:1. To each sample 100 µL of appropriately diluted sample extract and 900 µL of FRAP reagent was added and incubated at 37 °C for 40 min in the dark. In the case of the blank 100 µL of methanol was added to 900 µL of FRAP reagent. The absorbance of the resulting solution was measured at 593 nm by spectrophotometer. FRAP values were expressed as g Trolox/100 g DW of the sample.

**RESULTS**

**Morphological parameters**

Data presented table 1,2 indicate the propiconazole treatment the decrease the plant fresh weight compared with control plant where as plant fresh weight increased even when compared to Abscisic acid(ABA). The dry weight, pods and seed were decreased significantly by propiconazole as compared with control.

**Table. 1:** Effect of Propiconazole and Abscisic Acid on Number of pods and Number of seeds of *M.pruriens*.

	Number of pods	Number of seeds
Control	53.66±3.51	228±10.23
Propiconazole	74.2±5.03	345±7.89
Abscisic acid	50.8±3.00	276±9.21

\*Values are expressed in Mean±SD of three replicates.

**Table. 2:** Effect of Propiconazole and Abscisic Acid on Fresh weight (gm) and Dry weight(gm) of *M.pruriens*.

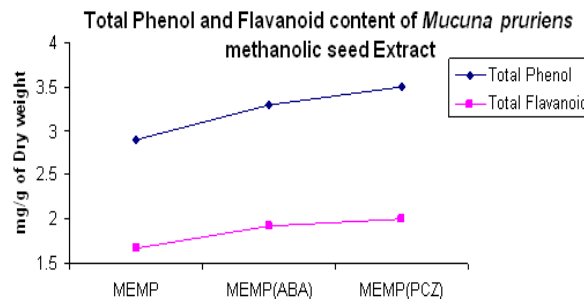
	Fresh weight(gm)	Dry weight(gm)
Control	299.59±5.21	173.42±4.79
Propiconazole	467.97±9.21	223.47±5.57
Abscisic acid	372.21±6.92	180.91±6.01

\*Values are expressed in Mean±SD of three replicates.

**Total phenol content**

The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. The total

phenolic content was higher in MEMP(PCZ) than MEMP(ABA) and control (Fig.3).



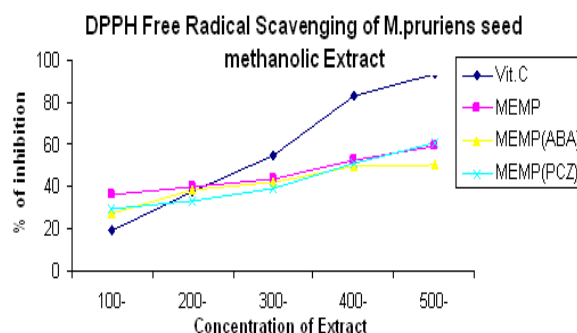
**Fig. 3:** Total phenol and Flavanoid content of methanolic extract of *Mucuna pruriens* seed. Values are represented three replicates of Mean ± SD.

**Total Flavanoids contents**

Recent studies have shown that, many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plants (Luo *et al.*, 2002). The total flavanoids in the MEMP higher, among the three extracts MEMP (PCZ) has shown higher phenolic content. (Fig.3)

**DPPH scavenging activity**

The free radical DPPH possess a characteristic absorption at 517 nm (purple in colour), which decreases significantly on exposure to radical-scavengers by providing hydrogen atoms or by electron donation. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Amarowicz *et al.*, 2004). From the analysis (Table 2), the radical-scavenging activity of the MEMP(PCZ) have found higher scavenging activity followed by MEMP(ABA) and control MEMP. DPPH scavenging activity was significantly correlated with phenolic flavonoids in extracts (Fig.1).

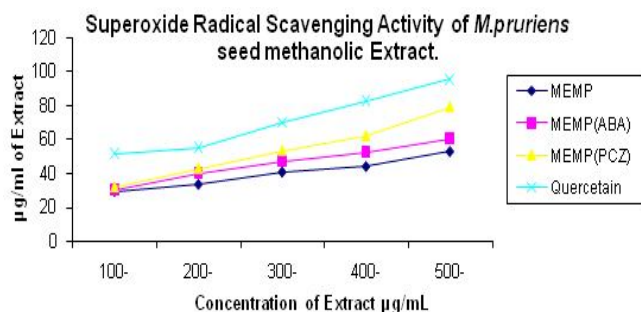


**Fig. 1:** DPPH radical scavenging activity of methanolic extract of *Mucuna pruriens* seed with standard Ascorbic acid. Values are represented three replicates of Mean ± SD. Concentration are displayed on logarithmic scale.

**Superoxide radical scavenging activity**

Superoxide (O<sub>2</sub><sup>•-</sup>) radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to the tissue damage and various diseases.

Fig. 2 shows the IC<sub>50</sub> of (•OH) scavenging effect by three extracts. Among the three the MEMP(PCZ) shows higher radical scavenging activity followed by MEMP(ABA) and control.(Fig.2)



**Fig. 2:** Superoxide radical scavenging activity of methanolic extract of *Mucuna pruriens* seed with standard Quercetain. Values are represented three replicates of Mean  $\pm$  SD.

### Ferric Reducing Antioxidant Potential

The reducing ability of MEMP was in the range of 69.63 of Fe (II)/g dry weight. The antioxidant potential in MEMP was higher in MEMP (PCZ) followed by MEMP(ABA) when compared to the control. It has shown higher ability to reduce 2,4,6-tripyridyl-s-triazine(TPTZ) – Fe (III) complex to TPTZ-Fe (II). The FRAP values for the methanolic extract of MEMP(ABA), MEMP(PCZ) were significantly higher than the BHT. The ferric reducing ability (FRAP assay) is widely used in the evaluation of antioxidant compound in dietary polyphenols.(luximon-ramma). Antioxidant activity is found to be linearly proportional with phenolic contents. Oktay *et al.* reported a strong positive relationship between total phenolic contents and antioxidant activity, which appears to be the trend in many plant species.(Table.3)

**Table. 3:** Radical scavenging activity (FRAP, Metal chelating of *Mucuna pruriens* seed Extracts).

	FRAP (g Trolox/100g of DW)	Metal chelating (%)
Control (MEMP)	69.63 $\pm$ 0.77	108.80 $\pm$ 3.80
MEMP (ABA)	62.22 $\pm$ 0.06	123.64 $\pm$ 2.99
MEMP (PCZ)	73.02 $\pm$ 0.45	138.62 $\pm$ 3.1

### Metal chelating activity

Reduction of Fe ions is an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. The presence of reductant (antioxidant) in the seed extract cause the reduction of Fe<sup>3+</sup> can be monitored.

The percentage of reducing ability of MEMP (PCZ) shows higher than MEMP(ABA) and control MEMP.(Table.3)

## DISCUSSION

Triazole compounds are systemic fungicide having plant growth regulating properties. They have the ability to alter the balance of important plant hormone including Gibberellic acid, ABA and Cytokinins (Hajjhashemi. 2007). Triazoles induced variety of

morphological and biochemical response in plant. It inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA (Fletcher. 2000). The reduction of shoot growth control and treated plants observed in the present study can be attributed to be senescence of shoot during the final phase of growth. The similar findings are also observed that the seedlings length were decreased with the increased concentration of propiconazole in red root (Bradley et al 2012). The reduced foliage weight observed was more pronounced in control than the ABA and propiconazole treated plants.

The present experiment investigate the antioxidant activity of methanolic extracts of *M.pruriens* seeds (MEMP), the seeds harvested from the Triazole treated *M.pruriens*. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen. Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants (Yanishlieva-Maslarova, 2001). The present experiment shows the MEMP (PCZ) had higher phenolic and flavonoid content. The high phenolic and flavonoid content in the MEMP (PCZ) may be responsible for its free radical scavenging activity. Free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxy radicals, peroxy radicals, and single oxygen. Herbal drugs containing free radical scavengers are gaining importance in treating such diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al.*, 2002). Fig. 1 shows the amount of each extract needed for 50% inhibition (IC<sub>50</sub>). IC<sub>50</sub> value of MEMP(PCZ) is 38.5 $\mu$ g/ml. The highest radical scavenging activity was showed by MEMP (PCZ) which is higher than that of MEMP (ABA), MEMP (control) and Ascorbic Acid. When the concentration increase the radical scavenging activity was increase. Increased inhibition activity shows the increase radical scavenging activity of the extracts. Therefore the antioxidant effects of *M.pruriens* was increased when the plant treated by propiconazole.

FRAP is the only assay that directly measures antioxidants in a sample. The other assays are indirect because they measure the inhibition of reactive species (free radicals) generated in the reaction mixture, and these results also depend strongly on the type of reactive species used. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric reaction (Halvorsen *et al.*, 2002). One disadvantage with the FRAP assay is that this assay does not react with thiols, because the reduction potential for thiols are generally below that of Fe<sup>3+</sup>/Fe<sup>2+</sup> half-reaction. The present observation study the FRAP assay, it indicates the MEMP (PCZ) has the rich reduction potential than compared to the other extracts. Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997). These processes can be

delayed by iron chelation and deactivation. Therefore, the ability of the extracts to chelate iron(II) ions was evaluated and expressed as % chelation capacity. The experiments shows the metal chelating power of different *M.pruriens* seed extracts, it's clear that chelating power of sample MEMP (PCZ) was higher as compared to the other extract MEMP(ABA) and MEMP(Control). In general several scientific finding shows that the Triazole compounds like propiconazole, paclobutrazol and tetraconazole etc, they were play the key role of increase the antioxidant activity of the plant (Kraus and Fletcher et al .,1994, Abdhul Jaleel *et al.*, 2007).

Paclobutrazol enhanced the free radical scavenging capacity of treated plants including the level of ascorbate and APX in Wheat seedlings (Berova *et al.*, 2002). In conclusion, the antioxidant potential of MEMP due to its strong hydrogen donating and metal chelating ability, as well as its effectiveness as a scavenger of hydrogen peroxide, superoxide and free radicals. In this case the Triazole treated plant seeds extracts shows more potent than the control due to the more antioxidant content that enhanced by triazoles compounds.

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