Effects of two botanical plant powder on the control of rice weevil, *Sitophilus oryzae* L. in stored rice grains

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Abstract : In the present study, powder prepared from the two indigenous botanical plants in Tamilnadu, India (*Acalypha indica* Linn. and *Phyllanthus amarus*) was tested for their efficacy on mortality of *Sitophilus oryzae* L. These plant materials showed the presence of various phytochemicals. The results revealed that all of the tested materials with some variations had lethal effects against the pest as compared with control. After 7 days of treatment of *S. oryzae* with 1.0 g of *A. indica*, 22.3 ± 1.67% mortality was obtained. However, the mortality rate was increased as 83.3 ± 1.41% after 28 days. At 2 g of *A. indica* treatment, mortality rate was increased as 92.1 ± 0.12% after four weeks. After two weeks, 3 g *A. indica* killed 94.6±1.41% *S. oryzae*. After 7 days of treatment of *S. oryzae* with 1.0 g of *P. amarus*, 20.3 ± 1.67% mortality was obtained. However, the mortality rate was increased as 86.4 ± 1.57% after 28 days. At 2 g of *A. indica* treatment, mortality rate was increased as 93.1 ± 0.12% after four weeks. After two weeks, 3 g *A. indica* killed 95.6±0.68% *S. oryzae* and mortality was 100±0.42% after 28 days of treatment.

***Key words: Rice grains, Rice weevil, Plants, Phytochemicals, Pest control*.**

I. INTRODUCTION

The rice weevil, *Sitophilus oryzae* L. is the important destructive, widespread major insect pest of cereals, especially stored cereals, throughout the world. Generally, female rice weevil oviposits directly into the seeds and completes developmental of larval stages inside the seeds and finally emerge as adult *Sitophilus oryzae* L. To control this organism, various synthetic insecticides have been used during storage of food grains. However, *S. oryzae* has been widely reported to develop resistance against various synthetic insecticides (Benhalima *et al*., 2004). Some plant families, particularly *Meliaceae*, *Asteraceae*, *Rutaceae, Piperaceae, Annonaceae, Verbenaceae* and *Labiateae* are good plant-derived insecticides (Isman, 1995). *Acalypha indica* is a common herb, and is mainly found in the backyards of houses and other places throughout the plains of India. These plants are very much used as expectorant, emetic, laxative, pneumonia, diuretic bronchitis, pulmonary tuberculosis and asthma. In homeopathy, this plant is mainly used in severe cough very much associated with bleeding complications in lungs, incipient phthisis and haemoptysis (Ghani, 2003).

In recent years secondary metabolites from plantshave been widely used as a source of novel bioactive agents (Okoli *et al*., 2009). Recently natural products from medicinal plants are being evaluated for presence of novel drugs with new modes of pharmacological action (Charles *et al*., 2011). The traditional medicinal methods, specially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries (Parastoo *et al*., 2012). *Sitophilus oryzae* are highly active in humid and warm area. It is well known that both the grubs and adults are very dangerous pests of stored products and stored grains namely rice, maize, wheat, barley, sorghum and lentil. Also, stored products such as, biscuits, corn flower, dried potatoes, beans, tamarind seeds, pumpkin seeds, millets etc.

In most of the cases they cause serious infestation i.e. a considerable amount of yield loss and spoiled more than they consumed. It was reported that, a larva of the Sitophilus oryzae generally consumes more than 14 mg grain/day and adult animal consumes 0.4 mg grain/day (Giolebiowska, 1969). Application of various synthetic chemical pesticides for grain protection is too common practice, but it may have serious drawbacks including attendant resistance, toxicity, and environmental pollution (Yusof and Ho, 1992). In these days, management of grains and stored product pests, using naturally available materials is the subject which received much more attention to overcome their problems because of their low mammalian toxicity and little environmental hazards (Isman, 1994).

The application of plant based products as grain protectants has been a traditional method in Indian villagers and attracted much more attention in the recent past. The medicinal plants possessing insecticidal as well as repellent properties with no or little mammalian toxicity are much in use. It was previously indicated that some medicinal plant extracts and powder have potent effect on stored grain insects such as toxicity and inhibit reproduction (Talukder and Howse, 1995). The medicinal plant based products prove superior to various synthetic chemicals as they are highly ecologically safe and easily biodegradable besides cheap. There has been some degree of achievements and success in the use of such medicinal plants. It is hoped that these concentred efforts shall eventually bring forth botanicals that can be widely used as alternate insecticides. Plants have been important source of drugs and insecticide in Indian system of medicine and other ancient systems in the rest of the world. The Indian tradition medicine system has an ancient heritage of traditional medicine.

II. MATERIALS AND METHODS

Experimental design

Acalypha indica Linn. and *Phyllanthus amarus* leaf powders were added separately to 25 gm of rice grain containing small boxes at various concentrations (1 gm, 2 gm and 3 gm). The rice grain and plant powder were mixed gently until the plant materials were uniformly distributed among the grains. In each box 10 adult beetles were introduced and covered with lid with holes to ensure ventilation. Mortality was registered after 7, 14, 21 and 28 days.

Preparation of plant extracts forphytochemical analysis

10 g air dried powder was weighed and transferred individually in 100 ml solvent such as, ethanol, aqueous, hexane, methanol and acetone for a day. The Erlenmeyer flask was kept on a orbital shaker at 150 – 200 rpm for 24 h. Then the sample was filtered using whatman's no 1 filter paper. The filtrates were further evaporated under reduced pressure and gummy residue was obtained with the help of rotary evaporator. The dried powder was further stored in a plastic vial at 4 °C until antimicrobial screening (Dabur *et al.*, 2004). The extraction procedure was repeated three times.

Phytochemical screening

In this study, phytochemical screening was carried out to find the presence of the active chemical constituents in the solvent extract (ethanol, ethyl acetate, chloroform, methanol and acetone) of nine medicinal plants. The following phytochemicals such as, flavonoids, alkaloids, phenolic compounds, tannins, steroids, total protein, carbohydrates, saponins, and glycosides (Parekh and Chanda, 2007b) were studied.

III. RESULTS

Phytochemical analysis of extracts from *P. amarus* and *A. indica* Linn

In this study, plant leaves were extracted with various organic solvents such as, ethanol, methanol, hexane, acetone and water. Methanol extract of *P. amarus* showed the presence of eight phytochemicals, whereas, acetone extract showed the presence of only four phytochemicals (Table 1). Likewise, *A. indica* Linn showed the presence of alkaloids, carbohydrates, steroids, glycosides, proteins, tannins, phenols, saponin and flavanoids in methanol extract. However, hexane showed the presence of only three phytochemicals (Table 2).

Table 1. Qualitative analysis of phytochemicals from *P. amarus* extracted with various solvents

Sl No	Tests	*P. amarus*				
		Ethanol	Methanol	Aqueous	Acetone	Hexane
1	Alkaloids	+	+	-	+	-
2	Carbohydrates	+	+	+	+	+
3	Steroids	-	-	-	+	-
4	Glycosides	+	+	+	-	+
5	Proteins	+	+	+	+	+
6	Tannins	-	+	-	-	+
7	Phenols	-	+	+	-	-
8	Saponin	+	+	-	-	+
9	Flavanoids	-	+	+	-	-

Table 2. Qualitative analysis of phytochemicals from *Acalypha indica* Linn. Leaves extracted with various solvents

Sl No	Tests	*Acalypha indica* Linn				
		Ethanol	Methanol	Aqueous	Acetone	Hexane
1	Alkaloids	+	+	+	+	-
2	Carbohydrates	+	+	+	+	+
3	Steroids	+	+	+	+	-
4	Glycosides	-	+	-	+	-
5	Proteins	+	+	+	+	+
6	Tannins	+	+	-	-	-
7	Phenols	-	+	-	-	+
8	Saponin	+	+	-	+	-
9	Flavanoids	-	+	+	+	-

Mortality of *S. oryzae* adults due to the activity of *A. indica* leaves powder

After 7 days of treatment of *S. oryzae* with 1.0 g of *A. indica*, 22.3 ± 1.67% mortality was obtained. However, the mortality rate was increased as 83.3 ± 1.41% after 28 days. At 2 g of *A. indica* treatment, mortality rate was increased as 92.1 ± 0.12% after four weeks. After four weeks, 3 g *A. indica* killed

94.6±1.41% *S. oryzae* (Fig. 1). After 7 days of treatment of *S. oryzae* with 1.0 g of *P. amarus*, 20.3 ± 1.67% mortality was obtained. However, the mortality rate was increased as 86.4 ± 0.12% after 28 days. At 2 g of *A. indica* treatment, mortality rate was increased as 91.4 ± 1.57% after four weeks. After three weeks, 3 g *A. indica* killed 95.6±0.68% *S. oryzae* and mortality was 100±0.62% after 28 days of treatment (Fig. 2).

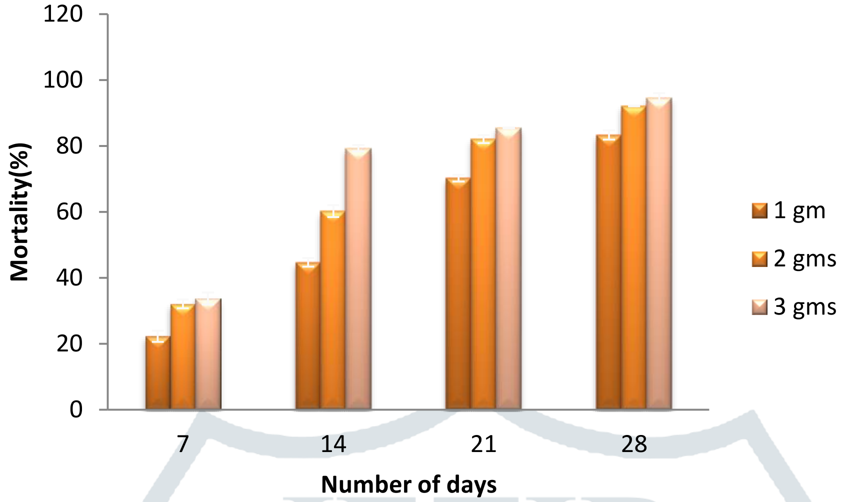

Fig. 1. Mortality response of *S. oryzae* adults to *A. indica* at various concentrations

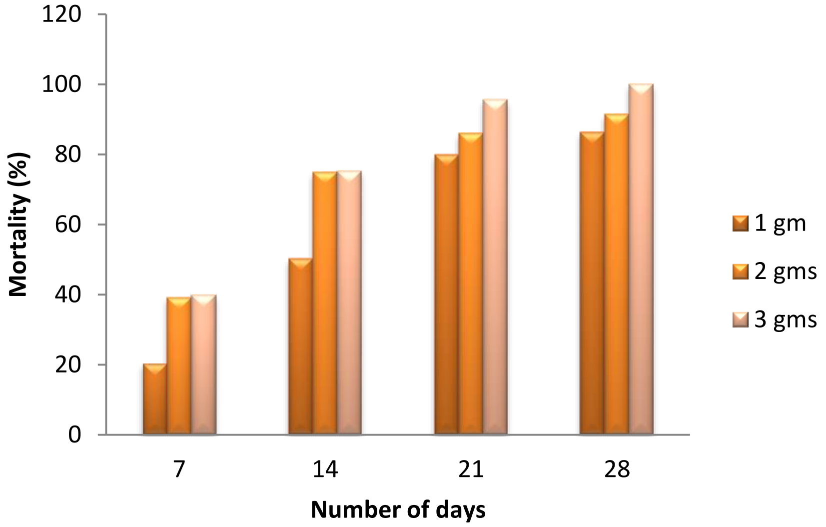

Fig. 2. Mortality response of *S. oryzae* adults to *P. amarus* at various concentrations.

Comparison between the effect of *A. indica* and *P. amarus* (2 and 3 gm) on *Sitophilus oryzae*

In the presnt study, after 7 days of incubation, 2.0 g *A. indica*, killed 32.1± 1.29% *S. oryzae*, however, 39.2 ±1.81% mortality was observed in the case of *P. amarus* powder. The mortality rate of *S. oryzae* was high when plant powder treated with *P. amarus* than *A. indica*. After 28 days of treatment, 91.4 ±1.57% mortality was observed when *A. indica* was treated with *S. oryzae*, 92.1 ± 0.12% mortalilty was registered when *P. amarus* was applied. After 7 days of incubation, 3.0 g *A. indica*, killed 33.7± 1.81% *S. oryzae*, however, it increased as 39.8 ±0.53% mortality was observed in the case of *P. amarus* powder. The mortality rate of *S. oryzae* was high when plant powder treated with *P. amarus* than *A. indica*. After 28 days of treatment, 94.6 ±1.41% mortality was observed when *A. indica* was treated with *S. oryzae*, 100 ± 0.42% mortalilty was registered when *P. amarus* was applied.

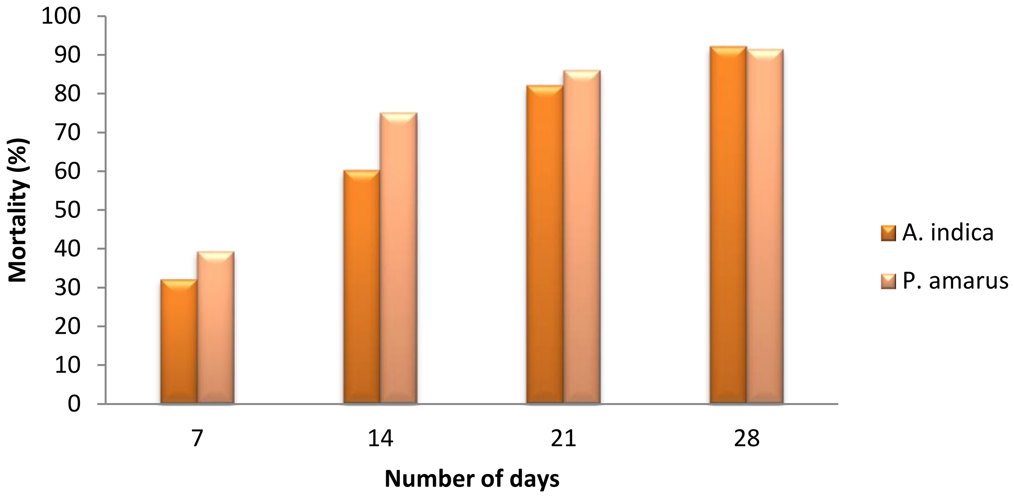

Fig. 3. Comparison between the effect of *A. indica* and *P. amarus* (2 gm) on *Sitophilus oryzae*

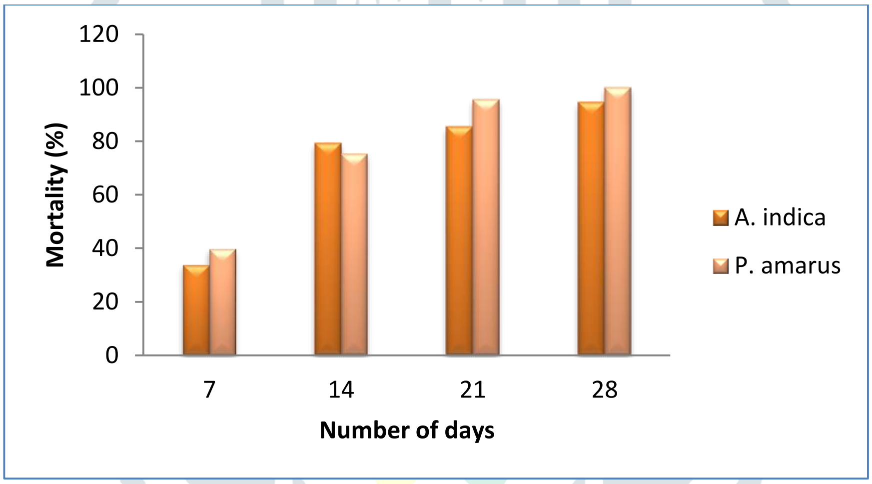

Fig. 4. Comparison between the effect of *A. indica* and *P. amarus* (3 gm) on *Sitophilus oryzae*

IV. DISCUSSION

The protection of stored products against attack by pests is essential in many countries, particularly those that do not have adequate storage facilities. Control of these pests relies on the widespread use of various synthetic chemical insecticides and fumigants. It has led to a number of serious problems such as environmental pollution, pesticide residue in food grains, pesticide resistance and toxicity to non-target organisms (Yusof and Ho, 1992). Therefore, the development of safer alternatives to conventional synthetic insecticides and fumigants is highly desirable. In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides.

In the present study, *P. amarus* and *A. indica*, was treated with rice containing *S. oryzae* and mortality rate was observed. After 7 days of treatment of *S. oryzae* with 1.0 g of *A. indica*, 22.3 ± 1.67% mortality was obtained. At 2 g of *A. indica* treatment, mortality rate was increased as 92.1 ± 0.12% after four weeks. After two weeks, 3 g *A. indica* killed 94.6±1.41% *S. oryzae*. Likewise, after 7 days of treatment of *S. oryzae* with 1.0 g of *P. amarus*, 20.3 ± 1.67% mortality was obtained. At 2 g of *A. indica* treatment, mortality rate was increased as 93.1 ± 0.12% after four weeks. After two weeks, 3 g *A. indica* killed 95.6±0.68% *S. oryzae* and mortality was 100±0.42% after 28 days of treatment. This is in support of a study as reported by Areekul *et al.* (1987) that water decoctions and volatile fractions of flower heads of *Tithonia diversifolia* showed 85 and 75% mortalities respectively of flies within the first hours after spraying which demonstrated high knockdown effects of aqueous extracts of *T. diversifolia* on the flies. Aqueous extract of *P. amarus* (5% concentration) showed some oviposition deterrent activities (more than 50%) against *Callosobruchus maculates*. In another study, ethanolic extracts of *P. amarus* at all the tested concentrations resulted in more than 70% mean mortality of the test samples, which may be due to knockdown effect of ethanol on insects and this suggested that *P. amarus* apart from its use in treating ailments also has insecticidal compounds present in it that are readily extracted in ethanol (Vanmathi *et al*., 2010). Phyto chemical constituents of coriander seeds have been studied extensively and its analysis has revealed the presence of polyphenols (rutin, caffeic acid derivatives, ferulic acid, galic acid, and chlorogenic acid), flavonoids (quercetin and iso quercetin) and β-carotenoids (Sergeeva, 1975). The essential oil obtained from the seeds contains α and β-pinene, camphor, citronellol, coriandrol, *p*-cymene, geraniol, geranyl acetate, limonene, linalool, myrcene, α and β phellandrene and α and β-terpinene. A large number of water soluble compounds have been identified that includes monoterpenoid glycosides, monoterpenoid glucose sulfate and other glycosides (Ishikawa *et al*., 2003).

Saljoqi *et al*. (2006) reported that *M. azadarach* was proved to be most effective against *Sitophilus oryzae* in the stored wheat grain. Similar trend was observed at 14 days after treatment, *M. azadirach* was recorded the highest mortality with 51.44 per cent among all the tested treatments. This was followed by *P. thyrsiflorus* with 31.91 per cent mortality. Whereas *A. indica*, *P. hysterophorus*, *V. trifolia and Z. acathopodium* gave the average mortality with 27.11%, 17.34 % and 12.53% respectively. Valladares *et al*. (2003) examined the antifeedent activity of an extract of senescent leaves of *M. azedarach* on nine insect species, including *S.oryzae*. The present results support the fiding of Khan and Marwat (2004) who reported that the leaves bark and seeds of bakain (*Melia azadarach*) and Ak (*Calotropis procera*) powder against lesser grain borer (*R. dominnica*). They tested that insect (*R. dominica*) was repellent from bakain's bark powder with 98.25% repellency followed by powder of Ak (*Calotropis procera*). In the presnt study, after 7 days of incubation, 3.0 g *A. indica*, killed 33.7± 1.81% *S. oryzae*, however, it increased as 39.8 ±0.53% mortality was observed in the case of *P. amarus* powder. The mortality rate of *S. oryzae* was high when plant powder treated with *P. amarus* than *A. indica*. After 28 days of treatment, 94.6 ±1.41% mortality was observed when *A. indica* was treated with *S. oryzae*, 100 ± 0.42% mortalilty was registered when *P. amarus* was applied. This is in consonance with the findings of Khanna *et al*. (2003) that showed that ethanolic extracts of aerial and root parts of *P. amarus* had some insecticidal activities against stored grain pest *Tribolium castaeneum*. Biocidal activity of extracts tested in this study against *M. bellicosus* via topical application test was not concentration-dependent as it resulted in an irregular patter of mortality rate, from the lowest to the highest concentrations.

In this study GC-MS analysis was carried out to determine active compounds from the methanolic extract of plant leaves. GC-MS spectrum of the methanol extract of *A. indica* showed the presence of six compounds, whereas *P. amarus* powder extract showed the presence of five compounds. GC-MS analysis has been frequently used to determine the active principle of phytochemicals from plants. In another study, the pytochemicals of *F. sycomorus* leaves were determined, and the compounds such as, 1, 2 benzenedicarboxilic acid, diisooctyl ester (45.06%), n-Hexadecanoic acid (7.67), 1H-pyrazole,4-Nitro (5.13), 3-Hexen-1-ol, benzoate,Z (4.57), oleic acid (4.30), hexanedioic acid, bis (2-ethyl hexyl) ester (4.15), methyl oleate (2.41), 3-buten-2-one, 4-(2, 6, 6-trimethyl-1-cyclohexen-1-yl) (2.08), 9-octadecenoic acid (Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester (1.79), benzene methanol (1.59), Cycloheptasiloxane,tetradecamethyl (1.38), Z, Z-3, 13-octadecadien-1-ol (1.31), 2-pentadecanone (1.27), 1-methylbicyclo [4.1.0] heptanes 1-methylnorcarane (1.06), L-linalool (1.04), cyclohexene (1.03) and methyl jasmonate (0.94) were determined (Romesh *et al*., 2013). In a study, GC/MS analysis of ethanolic extract of *Pergularia daemia* leaves confess the presence Hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23, Ethyl 9,12,15-octadecatrienoate (33.12 and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole (31.24). Qualitative phytochemical screening of the ethanolic extracts of the leaves revealed the presence of many compounds such as flavonoids, tannins, alkaloids, terpenoids, steroids and phenols. These phytochemicals showed potent insecticidal activity (Rukshana *et al*., 2017). These plants have a range of chemicals which can be isolated and used for pest control. The test plants being medicinal would yield environmentally sound chemicals having no harmful effects on the non target organisms.

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