The Potency of Six Medicinal Plant Extracts Against the Stored Grain Insect Pest *Sitophilus granarius* L.

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Abstract

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This study was conducted on six medicinal plants viz., *Vitex negundo* (leaves), *Xanthium strumarium, Caesalpinia bonduc, Mucuna pruriens, Moringa oleifera* (seed kernels), *Tagetes erecta* (petals) for their bio-insecticidal activity. The powders of various parts of plants were extracted using the MARS6 microwave acid digestion system. Three different concentrations (20, 30, and 40%) of plant extracts were tested against granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae) for their bio-insecticidal activities under laboratory conditions. The results of statistical analysis showed a good performance of all plant extracts, especially at the high concentrations of the extracts, where they showed different levels of insect mortality and their developmental rate was also reduced leading to significant reduction in insect numbers. The mortality rate ranged was 0-100% with *S. granarius* adults ($p < 0.01$). There was significant correlation ($R=1$) between mortality rate and the concentration of plant extracts. However, the extract of *Mucuna pruriens* with solvents (Acetone + Petroleum Ether) showed the highest mortality rate of 100% at the three concentrations used ($p < 0.01$), whereas, the lowest average mortality of 43.3% ($p < 0.01$) was observed with *Xanthium strumarium* and *Vitex negundo* extracts with solvents (Methanol + n-Hexane) compared with 0% mortality in the control. All plant extracts have revealed insecticidal as well as propitious protective effect on grains, and it can be selected as effective control treatment after proper dose formulation to prevent weevil infestation in stored grains.

Keywords: Bioinsecticide, stored grains, *Sitophilus granarius*, mortality, medicinal plants, MARS 6.

Introduction

Cereal crops are affected by a wide range of factors during its different stages from the field up to the consumer, such as moisture, heat, insects, rodents and mite, which negatively change the characteristics of the physical and technological properties of the seeds with measurable losses in either quantity or quality (Kiaya, 2014). Globally, the estimated crop yield losses due to the crop insect pests were around 20-40%. Similarly, the reported world economic losses due to the different plant diseases is around 220 billion US Dollars per year (Agrios, 2005). Bradshaw *et al.* (2016) also reported the annual loss due to the invasive insect about 70 billion US dollars.

Stored grains are infested with insect pests causing approximately 10-25% losses worldwide (Raja *et al.*, 2001), poor seeds germination (Santos *et al.*, 1990), and reduction in their market value (Hill, 1990). In the tropics, Weaver & Petroff (2004) reported 30%, with losses net value in USA over 200 million US Dollars, annually.

FAO (1998) reported around $13x10^6$ tons annual losses of stored grains caused by insects. Most of these insects are coleopteran species that thrive primarily on mould (Subramanyam & Harein, 1989; Viñuela *et al.*, 1993). The granary weevil, *Sitophilus granarius* L. is cosmopolitan in distribution. Both, adults and larvae cause damage to the stored grain (Fava & Burlando, 1995; Hill, 1990).

At present, the methods for controlling these pests are mainly based on the use of chemical insecticidal fumigants. The extensive use of synthetic insecticides for several decades led to long-term human health and environmental concerns, mainly because of their slow degradation rate in the environment and toxic residues, and the evolution of resistance to insecticides in insect pest populations (Isman, 2006) and consumer desire for pesticide-free grain (Arthur, 1996). According to estimates by the World Health Organization (WHO), there are around 200,000 deaths every year as a direct effect of poisoning with pesticides. Furthermore, the use of artificial chemicals nowadays became more restricted because of their high and acute toxicity, ability to create hormonal imbalance, spermato toxicity, long degradation period, carcinogenicity, and accumulation as residues in food (Dubey *et al.*, 2011; Feng & Zheng, 2007; Khater, 2012a).

Universally, the environment is facing critical threat from the massive use of chemical pesticides. Therefore, it was very important to search for safer methods to manage insect pests which affect crops and stored grains (Khanzada *et al.*, 2015; Sarwar & Sattar, 2012). Hence, it is important to find suitable safe, effective, biodegradable, eco-friendly and low-cost alternatives to control the stored grain insect pests (Isman, 2006, 2014; Gandhi *et al.*, 2010), and many of such alternatives are now available (Dales, 1996; Defagó *et al.*, 2009; Isman, 2006). Identification of effective biodegradable botanical bio-products which do not harm

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beneficial insects are now in progress (Haseeb *et al.,* 2004). There are many plants reported to possess pesticidal properties. These plants are a rich source of secondary metabolites, which might act on the insect physiological system and eventually control them (Daoubi *et al.*, 2005; Kim *et al.,* 2005). Locally available plants are currently in wide use to protect stored product against damage caused by insect infestation in many parts of the globe (Khater, 2012b). Monoterpenes and phenylpropenes found in these plants showed pesticidal activities have been tested for their larvicidal and bio-insecticidal property against several pests of stored products (Abdelgaleil *et al.*, 2009; Grodnitzky & Coats 2002; Lee *et al.*, 2003; Rice & Coats 2003; Cárdenas-Ortega *et al.*, 2005; Wuryatmo *et al.*, 2003; Duke *et al.*, 2000; Singh *et al.*, 2002; Lo Cantore *et al.*, 2009; Cristani *et al.*, 2007). The aim of the present investigation was to evaluate the bio-insecticidal effect of six Indian plant extracts classified as containing secondary bioactive compounds, extracted through MARS-6 instrument against the adults of *S. granarius*.

Materials and Methods

S. granarius rearing conditions

Adult insects of *S. granarius* were reared on wheat grains under laboratory conditions of 25±1°C temperature and relative humidity of 64±5%, with L:D (12 h:12 h) photoperiod in glass cylindrical containers closed by metallic net. The insects were provided by the Food and Environment Research Agency (FERA), Fera Science Ltd., National Agri-Food Innovation Campus and Hutton, York, YO411LZ, United Kingdom. Adult weevils obtained from these cultures were used in the bioassay experiment.

Plant Materials

Fine powder of six samples from different parts of plants brought from the rural area near Aurangabad city, Maharashtra state, India for use in this experiment. Leaves, petals, and seed kernels were air dried naturally, then crushed into small pieces and then pulverized into fine powder. These plants were: *Vitex negundo* (leaves), *Xanthium strumarium*, *Caesalpinia bonduc*, *Mucuna pruriens*, *Moringa oleifera* (kernels), and *Tagetes erecta* (petals).

Preparation of extracts using MARS 6 System

The microwave system MARS 6 based on adding two solvents at once to samples to obtain the extracts was used. 0.5 g of each powder was added to two solvents (10 ml of Methanol, and 10 ml of Hexane) in a glass tube and placed in system for 40 minutes. The same procedure and the same amount were used again with another two solvents, acetone and petroleum ether. The excess solvents were evaporated during 48 hrs. and the extract was filtered to remove residual plant materials. The extract was then diluted with distilled water to obtain different concentrations (20, 30, and 40%) used in the experiment (Al-Ghannoum & Karso, 2015; Singh, 1994).

Bioassay, experimental design and statistical analysis

Completely randomized design (CRD) was used in this experiment. Three concentrations (20, 30, and 40%) were prepared from extracts. Filter papers in petri dishes were treated with 10 ml of each extract. The control was treated with water only. Ten adults of granary weevil, *S. granarius* were brought from stock culture and placed in petri dishes (9 cm diameter) containing 5 g wheat grains and 5 g wheat flour. Adults were left at room temperature (24°C) and 65-70% RH. All these cultures were used to evaluate the efficacy of these plant extracts, and dead insects of *S. granarius* in each petri dish were noted and discarded. The results of adult mortality rate were determined 72 hours after exposure.

Results and Discussion

The differences among the plant extracts, extract concentrations, and solvents, tested were determined using analysis of variance test (One-way ANOVA). The data obtained showed significant variation ($P < 0.01$). There were highly significant differences in the influence of plants on the mortality rate of *S. granarius* adults ranged from 0 to 100% ($P<0.01$), with an average of 43.3-100% ($P<0.01$). The results obtained showed a significant difference due to different concentrations of the plant extract on adult's mortality rate (Figure 1). The mortality rate ranged from 0% for the low concentration of extracts (20%) and reached 100% for the high concentration of the extracts (40%) for all five plant extracts (Figure 2). The adult's mortality rate increased with increase in concentration. The results proved that the concentration 40% of all plant extracts was very effective in increasing adults mortality rate, which ranged from 70-100% (average 88.3%) with methanol + n-hexane and 90-100% mortality (average 96.7%) with the solvents acetone + petroleum ether (Figure 3).

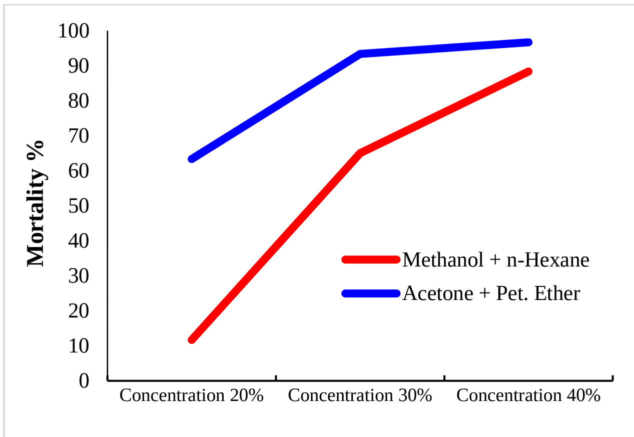

Figure 1. Average mortality rate *Sitophilus granarius* caused by three different concentration of plant extracts following the use of two groups of combined solvents. The first group included extracts obtained by using methanol and n-Hexane solvents, and in the second group, the extracts used were obtained by using acetone and petroleum ether solvents.

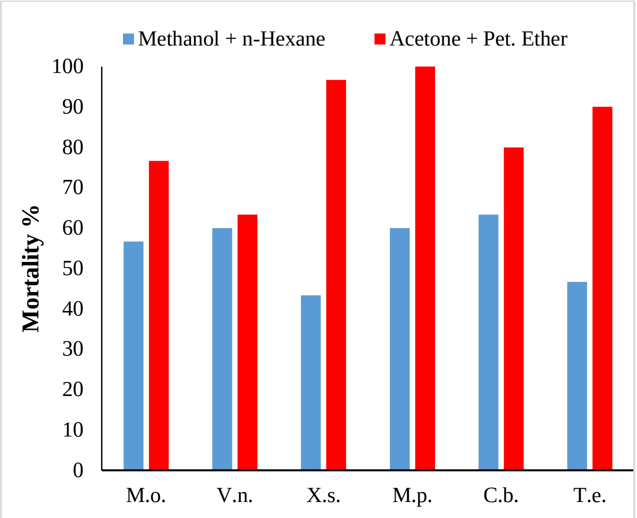

Figure 2. Average mortality rate of *Sitophilus granarius* following the use of six plant extracts by using two groups of combined solvents. The first group included extracts obtained by using methanol and n-hexane solvents, and in the second group, the extracts used were obtained by using acetone and petroleum ether solvents. Both groups showed their individual potential efficacy against the insect. Plants used were: M.o. (*Moringa oleifera*); V.n. (*Vitex negundo*); X.s. (*Xanthium strumarium*); M.p. (*Mucuna pruriens*); C.b. (*Caesalpinia bonduc*); T.e. (*Tagetes erecta*).

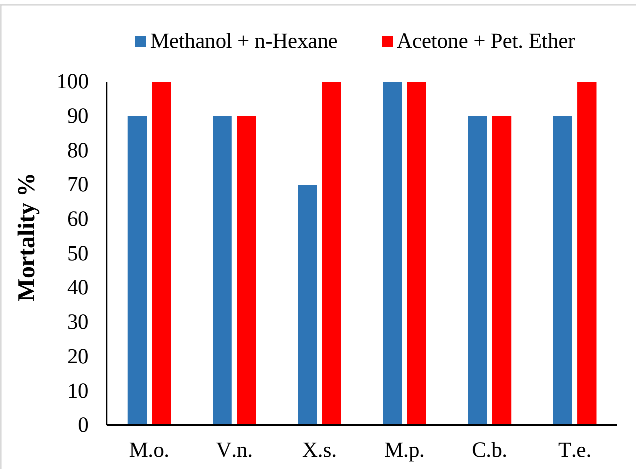

Figure 3. Mmortality rate of *Sitophilus granarius* caused by using six plant extracts and using two groups of solvents (methanol + n-hexane and acetone + petroleum ether solvents) at extracts concentration of 40%. Plants used were: M.o. (*Moringa oleifera*); V.n. (*Vitex negundo*); X.s. (*Xanthium strumarium*); M.p. (*Mucuna pruriens*); C.b. (*Caesalpinia bonduc*); T.e. (*Tagetes erecta*).

The results reported here are in conformity with an earlier report which evaluated the effect of different concentrations of *Nerium oleander* leaves extracts on *Tribolium castaneum* adults (Alghannoum & Karso, 2015). In the present study, significant difference was observed on the mortality rate when extraction was made by different solvents. The extracts obtained by the acetone + petroleum ether showed superiority in causing insect adults mortality rate of 63.3, 93.3, and 96.7% at 20, 30 and 40% extract concentrations, respectively (Figure 4), compared with 11.7, 65 and 96.7% mortality for using the solvents methanol + n-hexane with the same extracts concentrations, respectively (Figure 5). The minimum mortality rate of 0, 60 and 70% was observed with the same three plant extract *Xanthium strumarium* concentrations and using the solvents methanol + n-hexane, respectively. Whereas the highest mortality rate was obtained for the *Mucuna pruriens* plant extract and using acetone + petroleum ether solvents which led to 100% mortality for all three extract concentrations. These results are compatible with the results obtained earlier on the potential of *Mucuna pruriens* extract for its insecticidal activity against the adult beetles *Tribolium castaneum* (Hbst.) and cytotoxic activity against *Artemia salina* L. (Chhabi *et al.*, 2017).

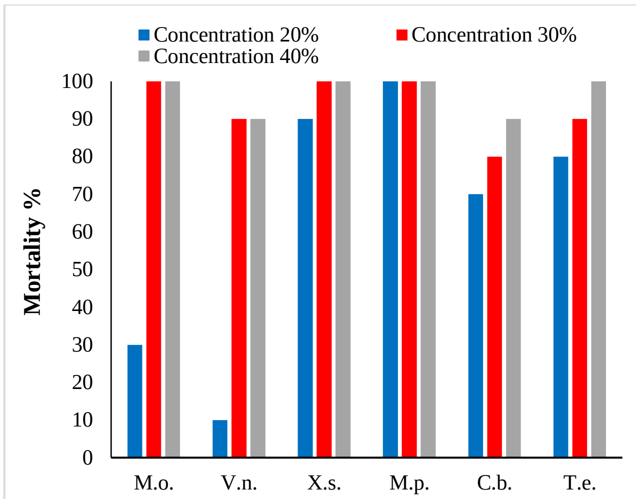

Figure 4. Average mortality rate of *Sitophilus granarius* following the use of six plant extracts with combined two solvents acetone + petroleum ether at 20%, 30% and 40% concentration. Plants used: M.o. (*Moringa oleifera*); V.n. (*Vitex negundo*); X.s. (*Xanthium strumarium*); M.p. (*Mucuna pruriens*); C.b. (*Caesalpinia bonduc*); T.e. (*Tagetes erecta*).

In addition to many medical benefits of *Mucuna pruriens* mentioned earlier (Lampariello, *et al.*, 2012; Yadav *et al.*, 2017), the plant contains a wide range of phytochemical constituents such as tannins, flavonoids, alkaloids and phenolic compounds which are responsible for different physiological effect and pharmacological activities (Deokar *et al.*, 2016). In the current investigation, mortality rate was significantly demonstrated for all six experimental plants extracts their efficiency against *Sitophilus granarius* insect pest. In many countries, the mixing of plant parts with grains is an ancient practice to manage stored grain insect pests (Kiruba *et al.*, 2008; Paul *et al.*, 2009). In such cases the larvae avoided feeding, and were not able to bore inside the grains, which can be due to the secondary metabolites present in the plants.

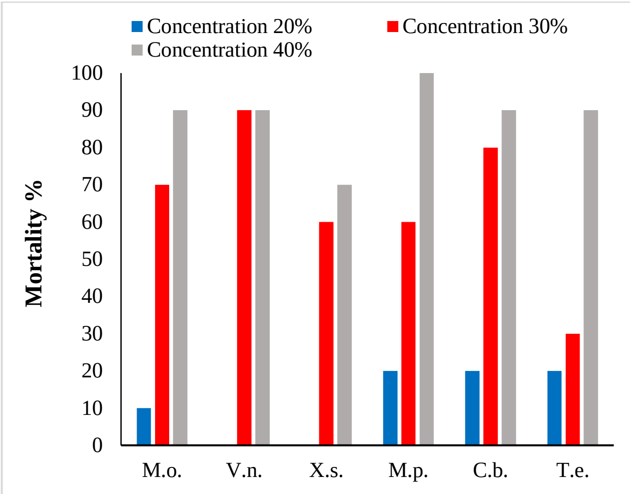

Figure 5. Average mortality rate of *Sitophilus granarius* following the use of six plant extracts and using methanol + n-hexane solvents at three different extract concentrations (20%, 30% and 40%). Plants used were: M.o. (*Moringa oleifera*); V.n. (*Vitex negundo*); X.s. (*Xanthium strumarium*); M.p. (*Mucuna pruriens*); C.b. (*Caesalpinia bonduc*); T.e. (*Tagetes erecta*).

In particular, the current six plants extracts were highly effective against insects, and contained compounds with insecticidal properties (Oelrichs *et al.*, 1983). Globally, herbal insecticides are attracting more attention as they are considered suitable and good alternative to chemical pesticides. Therefore, naturally occurring environmental friendly biopesticide could be an alternative for chemical pesticides. In support of our previous study, the extracts of six plants in this study have shown bio-pesticidal and grain protective effects along with reduced growth of *S. granarius* adults suggesting bio-efficacy of these six medicinal plant extracts to manage post-harvest grain losses during long term storage. Furthermore, the microwave digestion system MARS 6 was capable in providing fast, complete digestion for lower detection limits in a high-throughput environment as compared to Soxhlet's apparatus.

The results of the present study revealed that all the extracts obtained through MARS 6 system have shown different levels of insecticidal potential against *S. granarius*. The 40% concentration of all plant extracts was very effective in inducing adult's mortality of the insect pest.

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الملخص

جاولكار، ن.ب.، س.ب. زامبار وم.ع. الغنوم. 2021. فاعلية مستخلصات ستة نباتات طبية ضد آفة حشرة الحبوب المخزونة (*Sitophilus granarius* L.). مجلة وقاية النبات العربية، 39(4): 323–328. https://doi.org/10.22268/AJPP-039.4.323328

أجريت الدراسة على النباتات الطبية الستة التالية: أوراق *Vitex negundo*، بذور *Xanthium strumarium*، *Caesalpinia bonduc*، *Mucuna pruriens*، *Moringa oleifera*، وبتلات *Tagetes erecta*، وذلك لتقويم فعاليتها كمبيدات حيوية على حشرات الحبوب المخزونة. تم استخلاص المواد الفعالة من المساحيق الجافة لأجزاء مختلفة من النباتات باستخدام نظام الميكروويف MARS6 (تعمل درجات الحرارة المرتفعة والأوعية المغلقة والهضم الحمضي على تحضير العينات في وقت أقل من الطرائق التقليدية، وتستخدم حمضاً أقل، وتحتفظ حتى بالعناصر المتطايرة). تم اختبار ثلاثة تراكيز مختلفة من المستخلصات النباتية (20، 30 و 40%) ضد سوسة الحبوب *Sitophilus granarius* L. (Coleoptera: Curculionidae) لتحديد كفاءتها كمبيدات حيوية وذلك تحت الظروف المختبرية. أظهرت نتائج التحليل الإحصائي أداء جيداً لجميع المستخلصات النباتية، وبخاصة عند التراكيز العالية للمستخلصات، حيث أظهرت تفاوتاً في معدل نفوق الحشرات وانخفاض معدل نموها، مما أدى إلى انخفاض معنوي في أعداد الحشرات. تراوحت نسبة النفوق ما بين 0–100% عند البالغات ($p < 0.01$). أثبتت الدراسة وجود ارتباط معنوي ($R= 1$) وزيادة نسبة النفوق بشكل مباشر مع زيادة تركيز المستخلصات النباتية. ومع ذلك، أظهر مستخلص *M. pruriens* مع المذيبات Acetone + Petroleum Ether أعلى نسبة نفوق عند التركيزات الثلاثة 100% ($p<0.01$)، بينما بلغ أقل معدل نفوق 43.3% ($p<0.01$) لوحظ مع مستخلص *X. strumarium* و *V. negundo* مع المذيبات Methanol + n-Hexane مقارنة مع 0% عند الشاهد. أظهرت جميع المستخلصات النباتية قدرتها الوقائية كمبيدات كيميائية، ويمكن اعتمادها كخيار جيد بديل عن المبيدات الكيميائية عندما تحضر بالتراكيز المناسبة لمنع الإصابات الحشرية في الحبوب المخزنة.

كلمات مفتاحية: المبيدات الحيوية، الحبوب المخزنة، *Sitophilus granarius*، الوفيات، النباتات الطبية، MARS 6.

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